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VOLUME I NUMBER 4

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THE RELATION OF IODIN TO THE STRUCTURE OF THE THYROID GLAND.

DAVID MARINE, M.D. AND W. W. WILLIAMS, M.D.
[From the Laboratories of Pathology and Pharmacology of Western Reserve University.]
CLEVELAND, OHIO.

INTRODUCTION.

The analyses and experiments embodied in this paper comprise the second stage of the work on the nature of goiter, viz.: the relation of iodin to the structure of the thyroid gland.

In previous papers one of us[1] discussed the structure of the thyroid in its various phases in the attempt to formulate basic anatomic types to which the individual glands could be referred. It was found that the various types of changes are quite easily separated into four natural groups, viz.:

(1) Normal glands.

(2) Glandular hyperplasias, all degrees (parenchymatous hypertrophies).

(3) Colloid glands, goiter (all degrees).

(4) Complications supervening in any of the three preceding groups.

It has also been pointed out[2] that the anatomic changes observed in developing goiters are of such nature, and occur under such peculiar conditions, and can be controlled by such particular procedures, that we are forced to conclude that they are physiologic reactions to some deficiency, or compensatory hyperplasias.

Among the factors which might be connected with this deficiency, iodin was rendered most prominent by the observations of Halsted[3] on compensatory hypertrophy, by the discovery of Baumann[4] of the normal presence of iodin in the thyroid and by the vast accumulation of clinical and experimental evidence of the specific therapeutic effect of iodin in

1. Cleveland Med. Jour., 1907, vi, p. 45; also Johns Hopkins Hosp. Bull., 1907, xviii, 359.

2. Jour. Infect. Dis., Chicago, 1907, iv, 417; also Cleveland Med. Jour., 1908, vii, 105.

3. Halsted, W. S., Johns Hopkins Hosp. Rep., 1896, i, 373; also Med. Rec., N. Y., 1888, xxxiv, 368.

4. Ztschr. f. physiol. Chem., 1896, xxi, 319.

the hyperplasic or parenchymatous glands. We were, therefore, led to investigate further the relation of iodin to the gland structure. This work, so ably begun by Baumann[5] and others, undoubtedly lost much of its real worth by their attempts to apply the discoveries to analogous conditions in the human being before the difficulties and complicating conditions were fully recognized. Moreover, as the discovery of iodin came at the time when the chemistry of thyroid opotherapy was attracting the attention of other physiologic chemists who were more particularly interested in the organic constituents of thyroid proteids, the views of the above-mentioned investigators (Baumann, Oswald,[6] Roos[7]) as to the relation and importance of iodin in the proteid molecule met great opposition.[8]

R. Hutchinson[9] asserts that the activity of the thyroid is due to the two proteids of the colloid, but, after examining them in the light of Baumann's work, he found that both contain iodin, adding that one has only to realize the small amount of iodin (a few mgm.) to recognize that it can not play an active part. Concluding, he says: "The colloid matter then is the active substance—the part which iodin plays it plays by virtue of the form of combination in which it occurs in the colloid and not by virtue of its being iodin." In a second paper[10] he still considers iodin of secondary importance to the proteids in the therapeutic value of thyroid extract, and adds that if iodin in the colloid be increased ten times, as it is possible to do, it does not increase its activity.

To-day, however, one feature is generally agreed on, viz.: that the physiologic activity of the (colloid) thyreoglobulin is dependent on the amount of iodin contained in organic combination. Hunt[11] has recently arrived at similar conclusions in a novel way—that of testing the resistance of animals to a poison (acetonitrile) with and without the administration of thyreoglobulin.

In this work we have attempted to eliminate as many of the complicating, perplexing and uncertain factors as our present knowledge would permit, and to include all the factors which were open to observation and which at the time seemed to have a bearing on the structure or chem-

5. Ztschr. f. physiol. Chem., Strassb., 1896-7, xxii, 1; also Baumann and Roos, ibid, 1896, xxi, 480.

6. Ibid., 1897, xxiii, 265; also Inaug. Diss., Strassb., 1900.

7. Ibid., 1896, xxi, 19; also Virchow's Archiv., 1902, clxix, 444.

8. Notkin, Wien. med. Wchnschr., 1895, xlv, S. 824; 872; also Fränkel, S., Wien. klin. Wchnschr., 1895, viii, 842; also Med. Record, N. Y., 1896, xlix, 37.

9. Jour. Physiol., 1896, xx, 474.

10. Jour. Physiol., 1898, xxiii, 178.

11. The Journal A. M. A., 1907, xlix, 1323.

istry of the gland. With this idea in view, we chose to use the dog and have appended the full table of analysis, the special features of which will be pointed out under their appropriate headings.

The general views as to the etiology of goiter may be divided into two groups: (1) that it is due to an infecting agent (miasm) and (2) that it is the manifestation of a nutritional disturbance. The former had its period of greatest development parallel with the early period of bacteriology (1850-1885). Virchow, Hirsch and, in particular, Ewald have supported this view.

The latter view, that it is a nutritional disturbance, has received its greatest support from experimental investigations. The experiments, accidental, as it were, of the elder Kocher, and Reverdin,[12] of the total removal of the gland in man, were probably the most suggestive in the early period. The work of Munk[13] on the importance of the thyroid gland to the animal, pointing to conclusions which were so contradictory to the prevailing views[14], stimulated Halsted,[3] in the early days of aseptic surgery, to repeat them. Halsted's fundamental experiments and observations, followed by those of Horsley[15] and Edmunds,[16] have established several important general biologic facts, chief among which are: (1) that the gland is essential to normal body economy; (2) that partial removal is followed by compensatory hyperplasia of the remaining portion; (3) that myxedematous conditions follow the total removal of the thyroid gland alone, and (4) that it is possible to produce experimentally congenital (goiter) hyperplasia of the thyroid.

The introduction of thyroid feeding into the treatment of myxedema and later into that of goiter, in general, was particularly associated with the names of Murray[17] and Mackenzie[18] in England and Bruns[19] in Germany. Advances in our knowledge of the chemistry of the gland followed quickly on the discovery of its therapeutic merits and are in particular associated with Hutchinson,[9] Fränkel,[8] Baumann,[5] Oswald[6] and Roos.[7]

The results of all the investigations included in the above summary have tended to show (1) that the thyroid gland is an organ essential to

12. Quoted in report of the committee on myxedema, Trans. Clin. Soc., Lond., 1888, No. 21.
13. Sitzungsber. d. k.-preuss. Akad. d. Wissensch. zu Berlin, 1887.
14. Schiff, Arch. f. exp. Path. u. Pharmakol., 1884, xviii, 25.
15. Brit. Med. Jour., Lond., 1895, i, 111, 211; also Internat. Beitr. z. Wissensch. Med., 1891, i, 369.
16. Jour. Path. and Bacteriol., Lond., 1894-6, iii, 488.
17. Lancet, Lond., 1893, i, 1130.
18. Ibid., 123.
19. Beitr. z. klin. Chir., 1894, xii, 847.

the normal metabolic activities of the animal; (2) that it probably has a definite secretion; (3) that iodin is an essential constituent of this secretion, and (4) that the various anatomic changes occurring in the gland are results of or reactions against general nutritional disturbances rather than specifically thyroid in origin.

ANATOMIC CONSIDERATIONS.

The trite phrase, that "our knowledge of structure must precede our knowledge of function," seems particularly applicable to the study of the thyroid. Indeed, in our experience, the thyroid gland offers one of the most striking examples yet encountered of the relationship between variations in physiologic activity and in anatomic structure. In this work, a classification of the thyroid changes that is at once simple of application, well defined, and as free from vagueness as possible is absolutely essential. The present state of our knowledge offers no condition or factor, other than structure, that is sufficiently constant or uniform to allow of its use as a basis of classification. By examining large numbers of thyroids, it has been found possible to differentiate types to which the various grades and stages of the changes can be made to conform. Thus, by making as few subdivisions of these pure types as is possible the existing confusion in nomenclature may be obviated to a large extent. We also think that the anatomic standard of classification in experimental work on the thyroid will permit comparisons with other observers' work which, at present, is not possible. Such a classification will allow us to refer all phases of the subject under investigation to a fairly constant anatomic base-line and render possible an analytic study of these phases.

The material for this work consists of the thyroid glands of a series of over one hundred dogs taken without discrimination as to weight, age, sex or breed from the several department laboratories. Care was taken, however, to get the thyroids as soon as possible after the dogs were brought from the street in order that the findings might with justice be taken as an index of the normal street condition. As factors which might conceivably affect the condition of the thyroid, we observed the age, sex, breed, weight and state of nutrition. Records were made of all these conditions, though as regards age and breed gross errors are probable. The thyroid glands were weighed fresh; brief descriptions of color, consistency and visible colloid were noted; a small piece for microscopic

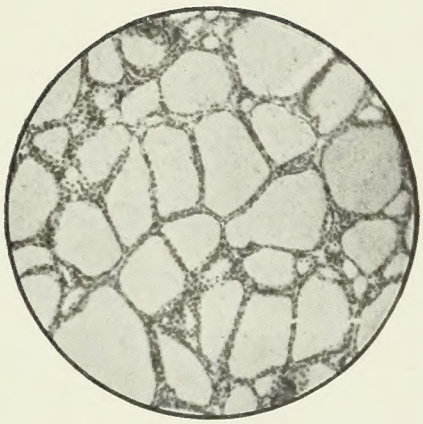

Fig. 1.—Normal thyroid, dog.

examination was then removed, and the gland reweighed and referred to Dr. Williams for iodin determination.[20]

As a result of an extensive experience with the anatomic appearances of the dog's thyroid, we have found that, while in general one can make fairly accurate determinations as to the type and even the grade of change from the gross appearance, there is always the chance for error; e. g., small glands may show quite marked hyperplastic changes, though, as a rule, the enlargement is proportional to the degree of glandular hyperplasia. Likewise, while the color and consistency and blood vessels, in general, conform to the glandular changes, reliance on them may be a source of error, as pointed out by Halsted.[3] The microscopic appearance, therefore, has been our sole final guide to the group or subgroup to which each gland was referred.

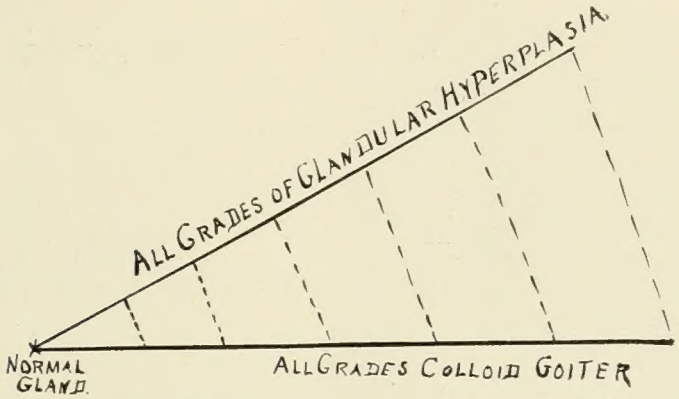

Fig. 2.—Showing relation of glandular hyperplasia to colloid goiter.

Barring complications, the three natural groups are, as previously stated: (1) normal glands, (2) colloid glands, (3) hyperplastic glands. Subgroups have been made only of Group 3—the glandular hyperplasias. It was further found necessary to make double reference to several of the glands, as *"normal X early," "colloid X early," "early X moderate,"* or *"moderate X marked."* These will be considered as intermediate groups. Brief anatomic descriptions of the groups and subgroups follow:

Group 1. *Normal Gland.*—The normal gland is similar, as to all its essential anatomic features, in all mammals examined. In herbivora, as a rule, the gland has coarser lobulations and more stroma. In the

20. The iodin determinations were made quite independently of the histologic work, and the anatomic groupings were made independently of the iodin determinations.

dog, the normal gland has a thin gray translucent capsule. The gland tissue is firm and of a yellow translucent color, due to the abundance of syrupy colloid. The blood and lymph vessels are very small. Microscopically, the acini are rounded, fairly regular spaces, lined by a single layer of flat cubical epithelium. The epithelial nuclei are small, flattened and darkly staining. The colloid is quite uniform and homogeneous in its staining reaction and sharply abuts on the lining epithelium. The stroma is very scanty. All glands conforming to this description were considered normal (Fig. 1). Glands to which there are double references are not included in the analytical tables. In our series there were but three[21] glands (A-38-87-98) which conformed to this rigorous standard—two from females and one from a male. All three dogs were in a fair state of general nutrition. Regarding age and breed there is nothing significant.

GROUP 2. *Colloid Gland.*—The colloid gland (goiter) in its uncomplicated form may be considered as an exaggeration of all the normal gland structures. Since it has been shown definitely by one of us that practically, if not quite all, colloid glands (goiters) have been preceded by a hyperplastic stage and are to be considered as the reverted and quiescent or as nearly the normal state as a gland can ever become which has once been the seat of a glandular hyperplasia, it then follows that we have to deal with all grades of the colloid condition, depending on the degree of hyperplasia preceding it. We think it necessary to consider all thyroids as colloid glands if they show any microscopic evidence of having had any grade of glandular hyperplasia preceding, no matter if their size, color, consistency and general appearance at the time of obtaining coincide with those of a normal gland. This view is shown more clearly in Figure 2.

To take, then, a moderate example of a colloid gland, the anatomic findings are as follows: The gland is symmetrically enlarged and very firm, the capsule is somewhat thickened and opaque, while the acini are large, well defined and filled with clear yellowish colloid. The tissue is translucent and not vascular. Microscopically, the arteries show an obliterating endarteritis similar to that occurring in the uterine or thymic vessels during involution. The colloid stains uniformly and densely and abuts sharply on the epithelium. The acinar epithelium is

21. The findings of but three absolutely normal glands out of the 113 specimens is due to the very rigid standard of normal adopted, viz., the seacoast type. Many ordinarily called normal were classified in the intermediate groups, i. e., under the double reference "normal x early" or "normal x colloid." These finer distinctions are only of value for scientific accuracy.

flat-cubical and arranged in a single layer. The nuclei are flattened, small and darkly staining. Depending on the degree of preceding glandular hyperplasia and on the duration of the colloid stage, there are more or less numerous sprigs of acinar epithelium jutting into the lumina of the acini, which are the remnants of the invaginations and plications of the epithelium during the hyperplastic stage. As may be inferred from Figure 2, there are stages in the process of reversion when it would be impossible to decide whether the histologic changes are to be considered progressive or reversive; hence, there is some difficulty of determining even histologically whether a given specimen ought to be considered with the colloids or with the hyperplasias.

Only glands conforming to the above anatomic description were considered as colloid in the analytical tables—the glands to which double references were made being classed in the intermediate group. There were eight cases (A-13, 25, 43, 48, 78, 80, 86, 90)—two females and six males. Relative to the state of nutrition, five were good or above and three were fair. It is significant that all cases occurred in middle-aged or old dogs. Our general impression is that in the dog sex has little or no influence on the structure of the thyroid (Fig. 3).

Group 3. *Glandular Hyperplasias.*—This group includes the most important thyroid changes, and it is with these changes in particular that this paper has to deal. Their greatest importance lies in the fact that they represent the primary changes[22] in the formation of all true goiters (functional hyperplasias), and hence must receive primary consideration in all inquiries into the nature of goiter. As pointed out above, there are all grades of these changes, from the normal gland to the most marked hyperplastic stage where there is scarcely any resemblance to thyroid at all. For this reason we have arbitrarily made three subgroups for analytical purposes. These groups are:

Subgroup 1. *Early Glandular Hyperplasias.*—This division comprises those glands which are but slightly removed from the normal. Several of the glands closely related to this subgroup are connected by double reference with the normal glands and included in the intermediate group of "normal X early." This is due to the fact that the changes are so slight that with fairness they must be considered doubly or not at all. These glands are not included in the primary analytical tables.

A typical example may be described briefly as follows: The gross appearance is nearly normal. The gland may perhaps be slightly en-

22. Virchow, Die Krankhaften Geschülste, iii, 4 et seq.

larged or of a pinkish hue if compared with a normal gland. The tissue is firm to the touch and on section colloid is visible. Microscopically, the acini are small, generally circular and regular. The colloid appears normal or nearly so as to staining reaction, but after some experience one can distinguish a slight vacuolization or at least a lessened staining intensity. The epithelium is regular and of a high cubical or low columnar type. There are here and there the very beginnings of invaginations and plications of the lining epithelium. The nuclei are slightly enlarged and darkly staining, but distinctly vesicular. There is no demonstrable increase in the stroma, but the vessels, particularly the finer ramifications, appear distinctly dilated. All glands conforming to this description are considered in this subgroup (Fig. 4). In our series there were nine cases—four females and five males (A- 39, 40, 57, 62, 65, 84, 88, 92, 103). All were considered to be in the middle period of life and in all the general nutrition was good. As to breed there is nothing of importance.

Subgroup 2. *Moderate Glandular Hyperplasias.*—This division likewise offers difficulties and opportunities for error when the attempt is made to bring into a single group. Hence many closely related glands are connected by double references with either colloids or early or marked glandular hyperplasias. The reason why some are connected by double reference with colloids is that occasionally one finds glands which appear to be reverting from a marked degree of hyperplasia, but at the time of taking were histologically much like moderate hyperplasias. Only undoubted cases were, however, included in the tables for chemical analysis.

Briefly, the anatomic appearances of a typical case are: The gland is moderately enlarged, the vessels are distinctly enlarged and somewhat tortuous, the capsule is thickened and the gland tissue is moderately soft and reddish in color. There may be visible colloid. Microscopically, the colloid is reduced, vacuolated, palely staining and granular, The acini are distorted by moderate ingrowths and plications of the epithelium. The acinar epithelium is high columnar in type, regular and arranged in a single layer. The nuclei are large, round, basal and vesicular. The stroma is slightly but diffusely increased and supports a rich network of dilated and enlarged blood vessels (Fig. 5). In our series there were nine cases (A-3, 6, 21, 34, 35, 60, 70, 76, 78)—eight males and one female. It is of interest to note that only three were considered in a good state of general nutrition. As to age and breed there is again nothing of significance.

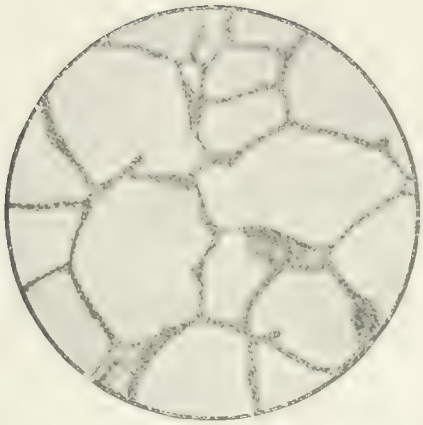

Fig. 3.—Colloid goiter. ×80.

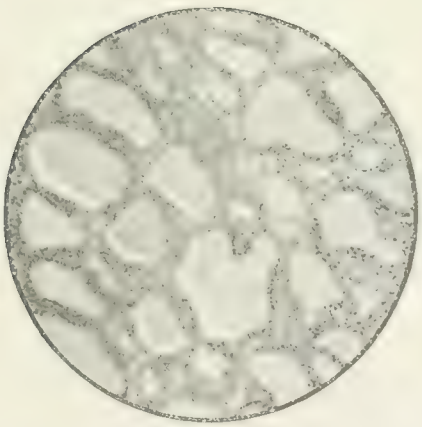

Fig. 4.—Early diffuse hyperplasia. ×80.

Subgroup 3. *Marked Glandular Hyperplasias.*—Glands in this stage of hyperplasia are more easily referred to an arbitrary subgroup for the reason that only the subgroup of moderate hyperplasias is to be differentiated, but here, too, an intermediate group is necessary.

The anatomic appearances of a typical example are as follows: The gland generally is considerably enlarged, but, as pointed out above, there may be very marked histologic changes without corresponding changes in the gross appearance. The capsule is thick and the vessels are much enlarged and tortuous. The lymphatic trunks are large, and, if exposed during life, are distended with a clear lymph-like fluid. On section the tissue is very vascular. It has a fleshy, opaque, gray-red appearance and a finely granular cut surface. There is no visible colloid. Microscopically, the stainable colloid is greatly reduced or absent, there being in its place only a small amount of pink staining granular material. The acini show extensive papillomatous ingrowths and plications of their lining epithelium, more or less effacing the acinar lumina. The epithelium is high columnar in type and arranged in a single layer. The epithelial nuclei are large, pale, round and basal. The cytoplasm is quite granular. The stroma is diffusely increased and is richly supplied with blood and lymph channels. There are also occasional collections of lymphoid cells in the stroma (Fig. 6). Only specimens conforming to the above description are considered in the analytical tables of this subgroup. In our series there are eighteen cases (A-2, 4, 10, 14, 15, 19, 23, 24, 28, 29, 41, 46, 47, 54, 67, 79, 99, 102)—ten males and eight females. As regards the state of nutrition, eight were classed as fair and ten as good. Relative to age the majority were young.

General Considerations of Sex, Age, Breed and State of Nutrition as Affecting the Structure of the Thyroid.

(a) *Sex.*—In this series there were forty-five females and fifty-eight males. (The remaining ten in the table were selected.) Sex does not appear to be a factor of noteworthy importance in the occurrence of thyroid changes in the dog; at least in the 103 cases included in this study no such relationship could be made out. From an experience with over 600 dogs our general impression is the same. This is quite different from the condition which obtains in man, and suggests that sexual states modify but slightly the activity of the thyroid of dogs as compared with man. Pregnancy, however, does induce hyperplasic changes similar to those occurring in man.

(b) *Age.*—Age is of more importance than is indicated in this series

for the reason that practically all the cases were from adult animals. From a series of over 600 dogs we have found that puppies are much more frequently affected, and, as a rule, the hyperplasia begins before the sixth month of extrauterine life. Authors generally are agreed that youth is the most important period in the human hyperplasias as well. Age is also a notable factor in the chemistry, as will be pointed out later. Age does not bear any important relation to normal glands. Colloid glands, however, are commoner in middle aged or old dogs without relation to sex. This conforms with the view that colloid glands (goiters) are reversions from hyperplasias.

(c) *Breed* in our laboratory class of dogs is an uncertain factor. Some authors think that, clinically, goiter is commoner in spaniels, but in our experience (using the histologic standard) we are unable to ascribe any importance to breed.

(d) The state of *nutrition* seems to be a factor of considerable importance both as a cause for and as an effect of the thyroid changes. All dogs with normal, colloid or early hyperplastic glands were found to be in a condition above fair,[22] while in the moderate and marked hyperplasias thirteen out of twenty-six were below this standard. Since fat is an apparent rather than a real index of nutrition, and since it is highly probable that goiter is a nutritional disturbance, it is also probable that great exceptions or variations will be noticed by the use of such a crude index, which, however, might be rationally explained if our knowledge of nutrition were more thorough.

CHEMICAL CONSIDERATIONS; IODIN DETERMINATIONS.

(1) The *method* used in all the determinations of the amount of iodin present in the glands and tissues investigated was based on that originally described by Rabourdin[23] and extensively used by Baumann and Roos[24] in their thyroid work. The basis of the method is the fact that the amount of iodin dissolved in chloroform can be determined by the depth of the pinkish tinge which it gives to the solution. Briefly, the method consisted in incinerating the tissue in a silver dish in the presence of an alkali and potassium nitrate, and after dissolving in water the iodin was liberated by acidulating with sulphuric acid; it was then shaken out with chloroform. In another flask a known amount of iodin was added to chloroform until the tint was the same as that in the first flask.

22. We have used in all of our work the following terms, poor, moderate, fair, good, excellent, using as the index the visible fat noted at autopsy.

23. Lieb. Ann., 76, S. 375.

24. Ztschr. f. physiol. Chem., Strassb., 1896, xxi, 481.

Baumann[25] asserted that the method was not very accurate, and Oswald[26] found that amounts under 0.3 mg. of iodin could not be detected when the material was incinerated in a silver dish by this method. He substituted a nickel dish and found that from 0.1 mg. of iodin he could recover 0.08 mg., while with larger amounts the percentage of loss was much less. These workers also found that the iodin-chloroform solution soon faded, and if the determination was not made quickly the result was very inaccurate and unsatisfactory. To overcome this disadvantage Anten[27] used carbon disulphid instead of chloroform, and found that the color was much more permanent.

Our method embraces these modifications, and was carried out as follows:

The fresh tissue is weighed and is then cut into small pieces and dried to an approximately constant weight at 110 C. The dried gland is powdered and 1 gm. is taken for the iodin determination.

In a nickel evaporating dish 2 gm. of pure potassium hydroxid are dissolved in 5-10 c.c. of distilled water, and to this the 1 gm. of powdered gland is added. This is slowly evaporated to dryness, then carbonized, and on the addition of potassium nitrate the mass is reduced to a clear flux, care being taken not to add an excess of the nitrate. After cooling, the flux is dissolved in 50 c.c. of distilled water and filtered. The filter is washed with distilled water until it no longer gives a precipitate with silver nitrate. To the filtrate is added 10 c.c. carbon disulphid, 3 c.c. of 10 per cent. sulphuric acid, and 2 c.c. concentrated sulphuric acid, saturated with nitrous acid. While adding the acids the flask is well shaken, and if iodin is present the carbon disulphid takes on a reddish-violet tinge.

Into another flask saturated sodium sulphate solution is poured until it is approximately equal in amount to the liquid in the first flask. To this is added 10 c.c. carbon disulphid, 3 c.c. of 10 per cent. sulphuric acid, and 2 c.c. concentrated sulphuric acid, saturated with nitrous acid. From a burette a potassium iodid solution containing 0.1538 mg. iodin per cubic centimeter (0.20 gm. exsiccated KI to 1 L. distilled water) is run in until the color of the carbon disulphid is the same as that in the other flask. Reading off the amount of potassium iodid solution used, it is a simple matter to determine the amount of iodin in the original solution.

25. Ztschr. f. physiol. Chem., 1896-7, xxii, 265.
26. Ztschr. f. physiol. Chem., Strassb., 1897, xxiii, 265.
27. Arch. f. exper. Path. u. Pharmakol., 1902, xlviii, 331.

The flasks used for the colorimetric method are similar to those described by Howald,[28] and have a capacity of 500 c.c. with the neck drawn out into a tube about 15 cm. long and 1.5 cm. in diameter and are glass stoppered.

Another method which was used as a control in fifteen determinations is that described by Fresenius.[29] The gland is treated exactly as has been described for the colorimetric method, and the liberated iodin is dissolved in carbon disulphid. This is then decolorized by adding from a burette a standardized solution of sodium thiosulphate, and the iodin determined by the amount of the thiosulphate solution used.

In comparing the results obtained by the two methods there occurred in one case a difference of 0.1 mg. of iodin, but, as a rule, the differences were well below this figure.

To determine whether preserved glands could be used for these determinations, parts of six glands were hardened in a 4 per cent. solution of formaldehyd in distilled water and then the iodin content was compared with that of a part of the gland treated in the usual manner. Three of the formaldehyd specimens had a slightly higher amount of iodin than the controls, but the difference between the average of those above and those below the mean was only 0.009 mg. iodin. It is, therefore, quite justifiable to use specimens preserved by formaldehyd.

(2) *Analysis of the Iodin Determinations.* Iodin determinations were made on 113 dogs' thyroids. The primary tables were made from the pure anatomic groups and subgroups, and hence include only the 67 cases there considered. This was done in the attempt to exclude all known conditions which might artificially modify the iodin content, and further, in order that the results might be taken to indicate the normal street condition. Many of the remaining 46 determinations will be utilized in the intermediate groups of Table 5 and in the analyses of the separate groups to show that the chemical conditions correspond with the anatomic relations. The remaining cases will be discussed under experimental observations.

(a) Beginning with Table 1 the following features are noted:

Excluding the colloid glands (goiters), it is found that the average amount of thyroid per kg. of body weight is practicallw the same (0.36 and 0.30 gm.) for the normals and early glandular hyperplasias, and that the moderate and marked hyperplasias show a progressive increase

28. Ztschr. f. physiol. Chem., 1897, xxiii, 212.

29. Traité d'analyse chemique, 6th ed., p. 406.

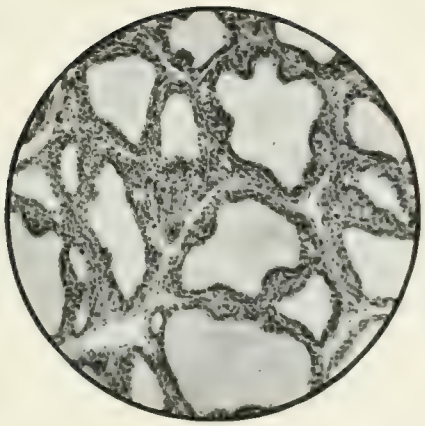

Fig. 5.—Moderate glandular hyperplasia, dog.

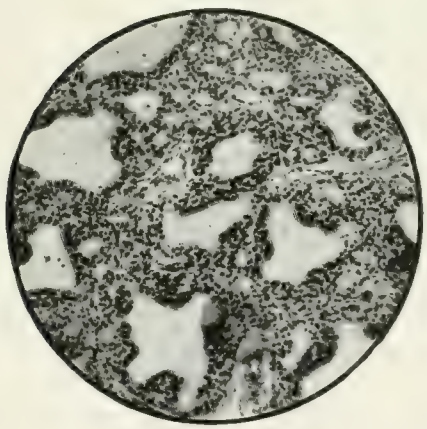

Fig. 6.—Marked glandular hyperplasia, dog.

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and W. W. Williams, M.D.

TABLE I—Amount of Thyroid Compared with Body Weight.

Character of Gland.	Number.	Weight of Dog. Expressed in Kg.			Weight of Thyroid Expressed in gm.			Dried Thyroid Expressed in gm.			Fresh Thyroid per Kg. Body Weight. Expressed in gm.			Percentage Water in Fresh Thyroid.		
		E.*	M.	A.	E.	M.	A.	E.	M.	A.	E.	M.	A.	E.	M.	A.
Normal.....	3	6.3 4.7	5.9	5.6	3.65 1.3	1.3	2.08	0.95 0.29	0.307	0.516	0.61 0.20	0.27	0.36	78 74	77	76
Colloid.....	8	13.6 5.4	9.1	9.1	20.45 1.25	7.32	8.54	5.57 0.115	1.49	2.13	3.19 0.21	0.73	1.07	91 68	73	76
Early.....	9	15. 5.	8.2	9.3	4.5 1.3	3.	2.76	1.184 0.22	0.65	0.643	0.45 0.15	0.30	0.30	83 72	74	77
Moderate.....	9	15.9 5.	8.2	8.56	22. 1.5	3.6	7.36	5.75 0.31	0.77	1.70	3.20 0.21	0.65	0.92	85 73	78	77
Marked.....	18	34.5 2.5	7.2	10.	77.9 3.2	7.1	16.45	18.2 0.44	1.36	3.59	13.2 0.23	1.135	2.34	88 69	80	80

* E = extremes; M = mean; A = average.

(0.92 and 2.34 gm. respectively). The colloids are intermediate between the moderate and marked hyperplasias (1.07 gm). It is also interesting to note, as pointed out by Oswald,[30] that the hyperplasias are reduced more in weight during the process of drying, i. e., contain a larger percentage of water than either the normals or colloids.

TABLE 2.—Iodin Content of Thyroid; expressed in Milligrams.

Character of Gland.	No.	Total Iodin.			Per Kilogram of Body Weight.			Per Gram of Fresh Thyroid.			Per Gram of Dried Thyroid.		
		E	M	A	E	M	A	E	M	A	E	M	A
		1.891			0.32			1.064			4.772		
Normal.. .	3		1.384	1.419		0 294	0.256		0.757	0.777		3.205	3.322
		0.984			0.156			0.512			1.990		
		6.675			1 236			1.039			3.608		
Colloid.. . .	8		3.013	3.232		0.359	0.402		0.406	0.459		1.816	1.985
		0.415			0.069			0.132			0.846		
		0.892			0.084			0.235			1.028		
Early. . . .	9		0.400	0.443		0.033	0.044		0.133	0.139		0.654	0.625
		0.107			0.012			0 063			0 243		
		1.679			0.173			0.137			0.854		
Moderate . .	9		0.399	0.539		0.061	0.069		0.076	0 078		0.345	0.368
		0.061			0.006			0.026			0.101		
		1.430			0.242			0.123			0.640		
Marked. . .	18		0.092	0.293		0.020	0.039		0.014	0.023		0.073	0.114
		0.013			0.002			0.002			0.008		

(b) In Table 2 the following points are brought out:

That the colloid glands (goiters) contain the most iodin—over twice as much as the normal glands—and that the hyperplasias show a decreasing amount, being least in the marked glandular hyperplasias, in spite of the fact that these glands show a progressive increase in weight, as shown in Table 1. As also noted in Table 1, the colloid glands (goiters) average more than four times the weight of the normal glands, and this accounts for the large amount of total iodin. But considering the amount of iodin per gm. of fresh thyroid, it is seen that the normal glands contain about 0.8 mg. per gm. and that there is a progressive de-

30. Ztschr. f. physiol. Chem., 1897, xxiii, 272.

crease reaching the minimum of 0.023 mg. per gm. in the marked hyperplasias.

There being but three normal glands included in this series, it is not justifiable to consider the base line as very definitely established, but the extremes being reasonably close together, it is probably not very incorrect.

Among the colloid glands (goiters) great variations are noted in the iodin content, due possibly to the strict histologic basis of classification[31]

TABLE 3.—Percentage of Iodin.

Character of Gland.	No.	Per Body Weight.[1]			Per Fresh Thyroid.[2]			Per Dried Thyroid.[2]		
		E	M	A	E	M	A	E	M	A
Normal	3	32	29	25	106	76	77	477	317	331
		15			51			199		
Colloid	8	123	38	41	104	41	46	332	181	195
		7			13			84		
Early	9	8	3	4	23	13	14	102	65	62
		1			6			24		
Moderate	9	14	6	6	14	8	8	85	34	37
		0.7			3			10		
Marked	18	24	1.5	3.8	12	1	2	64	8.5	11
		0.2			0.2			0.8		

1 = mgm. per kg. 2 = mgm. per gm.

(being irrespective of the size of the gland), and also to the possibility of the gland having just begun to undergo secondary hyperplasias (too early to be detected by any microscopic difference), in which case the iodin content is relatively low.

31. That is, all glands are considered as colloids if there is any histologic evidence that they had ever undergone any degree of hyperplasia no matter how slight. This classification would, therefore, be only of scientific value for the purpose of preserving uniformity.

(c) The percentage of iodin, compared with the body weight, is about 15 per cent. greater in the colloid than in the normal glands, whereas the hyperplasias contain about 20 per cent. less than the normal glands. Here, also, there is a tendency toward a progressive diminution of iodin depending on the degree of hyperplasia. A notable exception, however, is the group of moderate glandular hyperplasias, which contain a larger percentage of iodin than the early hyperplasias, and, as before stated, this can probably be accounted for by the difficulty of determining whether a gland is reverting to a colloid—when the iodin content is higher or developing a hyperplasia from a colloid base, in which case the iodin content is lower.

The percentage of iodin per gm. of fresh thyroid shows a progressive decrease from the normal glands through the colloids to the marked glandular hyperplasias.

Comparing the percentage of iodin in the normal glands with that in the colloid glands, it is seen that in the normal glands it is less per kg. of body weight and therefore absolutely less, while per gm. of thyroid it is much greater, showing that the colloid glands possess absolutely more iodin, although they are relatively poorer.

(d) Table 4 takes into account the extremes noted in Table 2 and is so arranged that each group is divided into those above and those below the average of the preceding group. As is to be expected, partly from reasons already given, the colloid glands (goiters) show the greatest extremes, but by comparing the iodin content of the glands above with that of the glands below the average content of the group preceding (normal), it is found that 75 per cent. have a greater absolute amount of iodin, but when the iodin is calculated per gm. of fresh thyroid, 89 per cent. fall below the average preceding (normal) content. All the early hyperplasias show a decrease below the colloid averages in total iodin and in iodin per gm. of fresh thyroid.

Allusion has been made to the difficulties encountered in grouping the moderate hyperplasias. The average of the total iodin in the nine cases of moderate hyperplasia shows 56 per cent. to be below the average for the preceding (early hyperplasias) group and all to be below when the iodin is calculated per gm. of fresh thyroid. Among the marked hyperplasias 78 per cent. are below the average total iodin content of the moderate hyperplasias and 95 per cent. below when calculated per gm. of fresh thyroid.

Considering all the groups, the extremes bear out the conclusions deduced from the means (Table 2), namely: that there is a progressive decrease in the iodin content per gm. of fresh thyroid from the normal glands through the hyperplasias.

TABLE 4.—Analysis of Extremes in Iodin Content noted in Table 2.

Character of Gland.	No.		Total Iodin.			Iodin per Kg. of Body Weight.			Iodin per Gm. of Fresh Thyroid.			Iodin per Gm. of Dried Thyroid.		
			E	M	A	E	M	A	E	M	A	E	M	A
Normal. . .	3		1.891			0.320			1 064			4.772		
				1.384	1.419		0.294	0.256		0.757	0.777		3.205	3 322
			0.984			0 156			0 512			1.990		
Colloid . .	8	Above preceding Average.	6* 6 675			5 1.236			1			1		
				4.000	4.059		0.397	0.585		1.039			3.608	
			1.753			0.351								
		Below...	2* 1 091			3 0.128			7 0.565			7 3.261		
				—	0.753		0.093	0.097		0.389	0.377		1.599	1.755
			0.415			0.069			0.132			0.846		
Early. . . .	9	Above...		0			0			0			0	
			9 0.892			9 0.084			9 0 235			9 1.028		
		Below...		0.400	0 443		0.033	0.044		0.133	0.139		0.654	0.625
			0.107			0.012			0.063			0.243		
Moderate..	9	Above...	4 1 679			5 0.173						1		
				0.796	0.969		0.115	0.111		0			0.854	
			0.608			0.061								
		Below...	5 0 399			4 0.039			9 0.137			8 0.519		
				0.123	0.195		0 013	0.018		0.076	0 078		0.318	0.308
			0.061			0.006			0.026			0.101		
Marked . .	18	Above...	4 1.430			3 0 242			1			1		
				0.818	0.920		0.135	0.156		0.123			0.640	
			0.615			0 091								
		Below...	14 0.473			15 0 035			17 0 042			17 0.184		
				0.080	0.114		0.014	0.016		0.014	0.016		0.069	0.083
			0.013			0.002			0 002			0.008		

* Figures indicate the number of glands.

TABLE 5.—Average Iodin Content of Pure and Intermediate Groups, Arranged in Anatomic Order.

Character of Glands.	No. of Cases.	Iodin per kg. of Body Weight.	Iodin per gm. of Fresh Thyroid.	Iodin per gm. of Dried Thyroid.	Fresh Thyroid per kg. of Body Weight.
Normals (pure)	3	0.256	0.777	3.322	0.27
Normals X colloids (intermediate)	1	0.218	0.480	2.480	0.46
Normals X early (intermediate)	7	0.054	0.173	0.881	0.27
Colloids (pure)	8	0.402	0.459	1.985	0 73
Colloid X early (intermediate)	4	0.181	0.217	0.914	0.79
Early (pure)	9	0 044	0.139	0.624	0.30
Early X moderate (intermediate	1	?	0.082	0.262	?
Moderate (pure)	9	0.069	0.078	0.368	0 65
Moderate X marked (intermediate)	9	0.096	0.055	0.283	1 52
Marked (pure)	...	0.039	0.023	0.114	1 14

(e) This table includes, besides the cases belonging to the pure histologic groups, those which are intermediate or which possess characters common to two groups, and excludes all cases which are complicated with neoplasm, hemorrhage, cystic changes, etc., and those operated upon or having received iodin in the laboratory or elsewhere.

It is compiled from averages only, and shows that the intermediate groups have iodin contents which fall between the iodin contents of the groups to which they are connected by double references. This table accentuates the fact brought out in Table 2 that the iodin per gm. of thyroid shows a progressive decrease from the normal glands through colloids to the hyperplasias, including the intermediate cases with double references.

(f) *Analyses of the individual cases of separate groups.* It will be noted that the extremes in the iodin content within the separate groups show such marked differences that it seemed unsafe to base conclusions solely on the averages. We compared, therefore, the iodin content with the anatomic changes of the individual members of each group.

Normal Glands.—The normal glands do not show very marked extremes, but as but three glands were considered, it is possible that a larger number might have shown greater variations. It is of interest, however, to note that in these cases the amount of iodin per gm. of thyroid varied inversely with the amount of iodin per kg. of body weight. An explanation of this fact might be that while the normal glands tend

to a more or less constant amount of iodin per gm. of thyroid, this constancy is not absolute, and when there is a greater quantity of thyroid tissue in one case the percentage of iodin will be less than in another case having less thyroid tissue.

As will be shown in the other groups, there seem to be comparatively wide limits in the iodin content between which there are no demonstrable histologic changes. On the other hand, there seems to be a fairly definite minimal amount of iodin per gm. of thyroid necessary for the maintenance of the normal histological appearance of the epithelium. If the iodin content falls below this minimal amount, hyperplastic changes in the gland follow. This minimal iodin content per gm. of thyroid is practically the same for the colloid and the normal glands as is shown in Table 5. In this table it is seen that hyperplastic changes have started when the iodin content falls below 0.173 mg. per gm. of fresh thyroid in the normal glands and to 0.217 mg. in the colloid glands.

Colloid Glands (goiters).—In the glands comprising this group there is, in general, an increase in the amount of iodin per gm. of thyroid associated with flattening of the epithelial cells and a decrease in the iodin in those glands having more cubical epithelial cells. As regards the relation between the amount of thyroid per kg. of body weight and the histologic changes—the smaller glands show evidence of epithelial activity (hyperplasia), while the larger glands show no such activity (cells very flat cubical).

Examining the cases further, it is found that six of the eight glands show that the increase in the amount of iodin per gm. of thyroid is coincident with a decrease in the amount of thyroid per kg. of body weight; in other words, the percentage of iodin is inversely proportional to the amount of thyroid, but since the amount of thyroid is very greatly increased, the total amount of iodin is also much increased, notwithstanding the fact that the colloid is relatively poorer in iodin than is normal colloid. This inverse proportion of iodin to the amount of thyroid agrees with that already noted in the normal glands, and our explanation is the same.

We have already pointed out that the colloid gland is as nearly normal as a gland can become after once having undergone a hyperplastic change, and it is of particular interest that the chemical findings should sustain this opinion which was based primarily on the histology, but which is now supplemented by the biologic facts that partial removal of colloids is followed by compensatory hyperplasia of the remaining por-

tion, and also, as shown by Baumann[32] for the normal glands, that compensatory hypertrophy occurs when iodin is restricted in the diet.

Oswald[33] has shown that in man and calves the iodin content in general varies with the amount of colloid in the gland, and also that in colloid goiters the percentage of iodin is decreased as compared with normal colloid. Our results with dog's colloid goiters agree with his findings.

Analyzing the glands still further, it is seen that, notwithstanding the comparatively low percentage of iodin in the colloid glands, the amount is always larger than that in colloids beginning to undergo hyperplasia. It seems probable that before a glandular hyperplasia can revert to a colloid, a necessary minimal amount of iodin must be present. Further, that reversion having once begun, the colloid accumulates more rapidly than iodin can be accumulated in the colloid (in the presence of a limited supply of iodin) without interfering with the physiologic requirements of the body at large, and, therefore, we have a colloid which is relatively poor in iodin as compared with normal colloid, although the absolute amount of iodin in the whole gland may be and is much greater than in a normal gland, and is, if our interpretation be correct, necessarily greater than the normal physiologic needs of the organism. But in the presence of a large excess of iodin the colloid of a reverting hyperplastic gland is capable of assimilating as much iodin as normal colloid. In other words, a certain amount of iodin is necessary before reversion can occur at all. If this supply of iodin be only slightly in excess of the physiologic needs, then the large amount of colloid which is formed will be relatively poor in iodin, although the total iodin in the gland may be and is actually greater than that present in a normal gland. If, on the other hand, the gland reverts in the presence of a larger excess of iodin, the colloid which is formed will be rich in iodin, approaching or equalling the iodin content of the colloid of normal glands.

"Colloid X Early" Glandular Hyperplasia.—This group shows that the amount of colloid in the gland decreases as the amount of iodin increases; that the colloid decreases as the gland becomes smaller, and that the iodin per gm. of fresh thyroid decreases as the gland becomes larger. These conclusions hold good for three out of four glands—the fourth gland was reverting from a marked hyperplasia, while the others were in the early stage of secondary hyperplasia engrafted on colloid bases.

32. Ztschr. f. physiol. Chem., 1896-7, xxii, 14.
33. Ztschr. f. physiol. Chem., 1897, xxiii, 265.

"Normal X Early" Glandular Hyperplasia.—In this group, roughly speaking, the amount of iodin per gm. of fresh thyroid decreases as the amount of thyroid per kg. of body weight increases. Moreover, the amount of colloid decreases as the amount of thyroid per kg. of body weight increases, and, finally, in two-thirds of the glands the amount of iodin decreases as the colloid decreases. Altogether the results are neither striking nor conclusive.

Early Glandular Hyperplasias.—The amount of iodin per gm. of fresh thyroid decreases as the amount of thyroid per kg. of body weight increases. There is no apparent relation between the amount of iodin and the amount of colloid present in the gland, nor does the degree of hyperplasia bear any marked relationship to any of the other factors. The amount of thyroid per kg. of body weight varies inversely with the amount of colloid in a fairly constant manner—the only striking exception—A-103—approaches histologically the next group.

Moderate Glandular Hyperplasias.—With the exception of three glands, the amount of iodin per gm. of fresh thyroid varies directly with the amount of thyroid per kg. of body weight. Two of the three exceptions have such an unusually large amount of thyroid per kg. of body weight that they suggest some other pathological changes. Roughly speaking, the amount of iodin increases as the amount of colloid increases. No relation seems to exist between the amount of colloid and the amount of thyroid per kg. of body weight. The epithelial differences are not sufficiently characteristic to permit drawing any conclusions.

"Moderate X Marked" Glandular Hyperplasia.—In seven out of the nine glands the iodin tends to vary inversely with the amount of thyroid per kg. of body weight. Also the colloid tends to vary inversely between the amount of colloid and the amount of thyroid per kg. of body weight.

Marked Glandular Hyperplasias.—In general the amount of thyroid per kg. of body weight tends to vary inversely with the amount of iodin, but to this there are some exceptions. There seems to be no relation between the iodin content and the amount of colloid, though the amount of visible colloid is so small that accurate estimations are impossible. The amount of colloid tends to run parallel with the amount of thyroid per kg. of body weight, but with rather numerous exceptions.

Summary of the Relation of the Iodin Content, Size of Thyroid, and Quantity of Colloid.

In Table 6 the amount of iodin is found to vary inversely with the amount of thyroid per kg. of body weight, and the amount of iodin in the colloid varies inversely in the normal and colloid glands. In the

TABLE 6.—A. Based on the Averages from Table 5.

Character of Glands.	Iodin to Thyroid per kg. of Body Weight.	Iodin to Colloid.	Colloid to Thyroid per kg. of Body Weight.
(1) Normals and colloids	Inverse	Inverse	Direct
(2) From normals through hyperplasias	Inverse	Direct	Inverse

B. Based on the analyses of the individual glands of the separate groups and intermediates, as described in the preceding section.

Character of Glands.	Iodin to Thyroid per kg. of Body Weight.	Iodin to Colloid.	Colloid to Thyroid per kg. of Body Weight.
(1) Normals	Inverse	Inverse	Direct
(2) Colloids	Inverse	Inverse	Direct
(3) Normal X early	Inverse	Direct	Inverse
(4) Early	Inverse	No relation	Inverse
(5) Moderate	Direct (?)	Direct	No relation
(6) Moderate X marked	Inverse	Inverse (?)	No relation
(7) Marked	Inverse	No relation	Direct

hyperplasias the amount of iodin also varies inversely with the amount of thyroid per kg. of body weight, but the amount of iodin in the colloid varies directly with the colloid. In the normal glands and in the colloids the amount of colloid in 1 gm. of fresh thyroid compared with the amount of thyroid per body weight seems to vary directly, while in the hyperplasias it varies inversely.

Comparing these conclusions with those of the analyses of the individual glands of the separate groups, a general agreement is found; that is, these changes are evident not only when whole groups are compared, but when the gradations of the individual members of the groups are compared. The agreement is not, however, absolute in detail. From Table 6-B it will be seen that the results in the separate groups agree with those of the averages in eight instances and are contrary in three. Similarly, within each group, individual exceptions are noted. It appears evident, therefore, that other factors than iodin are concerned which are not taken into account in our observations. In some of the cases at least the factors were plainly accidental, such as hemorrhage, cysts, etc.

However, the very striking fact that the same general laws hold good for the individual cases as well as for the whole group proves that iodin is the predominant objective factor.

(3) *Effects of Weight, Age, Sex, Nutrition and Breed on the Iodin Content.*—(a). The amount of normal thyroid per kg. of body weight is about 0.35 gm., while in our groups it averaged 1.07 for the colloids, 0.30 gm. for the early glandulars, 0.92 gm. for the moderates, and 2.34 for the marked glandular hyperplasias.

(b) Age in relation to iodin content. Thirteen of the sixty-seven glands used in the tables were from dogs under 6 months of age, and show a distinctly low percentage of iodin. In this connection it might be recalled that Baumann found that children at birth rarely had any iodin in the thyroids and that with years the quantity progressively increased, being greatest between the ages of 25 and 45 and diminishing again in old age. Six glands in our series were marked "old," but show no definite change.

(4) Nutrition is probably of more importance than is here indicated, since practically all dogs were in good health. As a rule, the very marked cases of glandular hyperplasia occur in the young and are associated with a greatly reduced iodin content or none at all.

(5) Breed is an indeterminable factor in our cases.

(6) Intercurrent factors. Two pregnant bitches were included in our series; these showed no definite change in iodin content.

EXPERIMENTAL OBSERVATIONS.

(1) *Effect of Feeding Iodin Containing Substances* (Table 7).—In the six cases one lobe was removed as a control, and then different iodin-containing substances were administered in known quantities to ascertain the rapidity of assimilation and the amount stored, as well as to determine what changes occurred in the histologic structure of the gland and the rapidity of these changes. The iodin was administered in the following forms: Saturated alcoholic solution of iodin; syrup of the iodid of iron; desiccated thyroids (commercial); fresh sheep thyroids (all degrees of glandular hyperplasia); full mixed diet (constant factor in all).

As regards the changes in the size of the gland, all the hyperplastic glands show a remarkable reduction in the weight of the remaining lobe, which conforms with the vast clinical observations on man and animals; and, within limits, this occurs without apparent relation to the forms in which the iodin is administered. On the other hand, the normal and colloid glands show no diminution in size.

TABLE 7.—Experimental — Iodin Feeding.

No. of Dog.	Weight of Dog. Kg.	Weight of Fresh Thyroid.	Weight of Dried Thyroid.	Character of Gland.	†Total Iodin.	Iodin per Kg. of Body Weight.	Iodin per Gm. of Fresh Thyroid.	Iodin per Gm. of Dried Thyroid.	Remarks.
26a	2.4	0.24	0.04	Normal . .	0.092	0.038	0.380	2.3	15 gtt. sat. alc. sol. iodin in 13 days; 27 days before death.
26b	4 0	0.305	0 06	Normal . .	0.215	0.053	0.705	3.467	
51a	3.2	22.1	4 47	Colloid. . .	3.16	0.987	0 138	0.707	6 5-gr. thyroid tablets in 3 days; 2 days before death.
51b	3.0	23.1	5.85	Colloid. . .	5.669	1 889	0 245	0.969	
16a	5.0	4.15	0.714	Moderate .	0 061	0.012	0.014	0.086	65 5-gr- thyroid tablets in 13 days; 14 days before death.
16b	3.2	2.7	0.61	Colloid, . .	1.061	0.331	0.393	1.739	
18a	5 4	7.5	1.63	Moderate .	0 425	0.076	0.056	0.261	545 gms. fresh sheep thyroid in 10 days; 8 days before death.
18b	3.18	4.75	1.25	Colloid. .	6.018	1.889	1.265	4.184	
52a	2.7	3 6	0.52	*Moderate Marked	0 215	0.079	0.057	0.412	15 gtt. sat. alc. sol. iodin in 3 weeks; 2 days before death.
52b	2.8	2.0	0.51	Colloid. .	1 63	0.563	0 815	3.196	
55a	7.27	72.0	13.15	*Moderate Marked.	0.61	0 083	0.008	0.054	7 gtt. sat. alc. sol. iodin in 7 days; 1 day before death; died suddenly; embolus.
55b	6.36	57 2	11.97	Moderate .	16 746	2.633	0.292	1.399	

* Moderate marked glandular hyperplasia.

† Iodin is expressed in milligrams.

The histologic changes in all the hyperplastic glands show a return to the colloid type. In our experience this reversion takes place in about two or three weeks, when there is an excess of iodin present. None of the cases, including the normal glands, show any hyperplasic changes even though the period of forty days, which Halsted advised to allow for the occurrence of hyperplasia, was permitted to elapse.

The total iodin in the control thyroid was invariably much less than the total iodin in the lobe removed after feeding iodin. The amount of iodin assimilated does not seem to be so much dependent on the form in which it is administered as on the amount of glandular tissue present.

It is interesting to note that the iodin content of the colloid of normal glands can be much increased, and also that the colloid from colloid goiters can have its iodin content increased to the same extent. In one dog (A-17), not included in the table, one thyroid lobe was removed during pregnancy. It weighed 0.7 gm. and histologically showed early hyperplasia. The remaining lobe was removed at the end of pregnancy, the dog having received 0.2 gm. of iodin. The lobe weighed 0.65 gm., and histologically was a pure colloid. The iodin in this gland per gm.

TABLE 8.—Experimental—Without Iodin Feeding.

No. of Dog.	Weight of Dog.	Weight of Fresh Thyroid.	Weight of Dried Thyroid.	Character of Gland.	Total mg. Iodin.	Iodin per Kg of Body Weight.	Iodin per Gm. of Fresh Thyroid.	Iodin per Gm. of Dried Thyroid.
27a	1.85	0.25	0.07	Normal.	0 062	0.033	0.248	0 886
27b	1.53	0.15	0.025	Early.	0.038	0 024	0.253	1 520
63a	12.7	14.25	3 41	Colloid.	4.249	0 334	0.298	1.246
63b	12 7	14.0	4.03	Colloid.	1.302	0.102	0.092	0 323
95a	5.9	5.1	1 2	Colloid.	1.284	0 218	0 251	1.076
95b	5.67	3 4	0.77	Colloid.	0.554	0 097	0.163	0.719
64a	2.7	2.25	0 36	Early.	0.108	0 005	0 051	0.300
64b	4.1	2.3	0.41	Colloid.	0 954	0.332	0.415	2.326
94a	2.15	1.5	0.28	Early.	0.184	0.085	0.122	0.657
94b	1.95	1.0	0.21	Early.	0.215	0.110	0.215	1 024
74a	5.44	3.6	0.5	Moderate.	0.261	0 048	0 072	0.521
74b	6.35	2.8	0.55	Early.	0.799	0.125	0.275	1 452

was 1.093 mg., which is above the average for a normal gland. Specimens of liver and muscle showed no trace of iodin.

(2) *Effect of Removing One Lobe Without Administration of Iodin* (Table 8).—In the six dogs one lobe was removed as a control, and the dogs were then kept on the usual laboratory diet (bread, cooked meat, salt and water) without the direct administration of iodin. All show reduction in the size of the remaining lobe, manifested most particularly in the hyperplasic glands, but not as great as is noted in the dogs which had received iodin. The colloids also show some reduction in size, but to a less extent.

Dog 27a was used as the control for 26a. They were sisters and both had normal control lobes. The second lobe of 27a showed the usual compensatory hypertrophy (Halsted), while the second lobe of 26a remained normal in type, though it grew somewhat in size.

The colloid glands showed no histologic evidence of hypertrophy, although chemically they did show evidence (i. e., a reduction in the iodin content). All the hyperplasic glands showed some evidence of reversion, but it was very slight compared with that occurring in the dogs which had been fed iodin. The normal and colloid glands both fell off rapidly in their iodin content, in sharp contrast to those fed iodin. On the other hand, however, the hyperplasic glands showed a slight in-

crease in the iodin content, though much less than in those where iodin had been fed.

This suggests that keeping the dogs on the same diet, the hyperplasic (hyperactive) glands are more capable of taking up iodin than are the more inactive normal or colloid glands.

When we consider dogs 26 and 27 along with dogs 7, 9 and 11, and in connection with the general experimental evidence thus far obtained from various investigators, it further suggests that there is a minimal normal amount of thyroid tissue and a minimal normal amount of secretion necessary for the normal body economy, which is dependent on the available supply of iodin, i. e., the greater the supply of iodin the less the necessary amount of thyroid. This suggestion is further supported by the fact, first pointed out by Halsted, that it is possible to produce goitrous pups by the partial removal of the mother's thyroid, when she is kept on the ordinary diet. We have in addition, experimentally shown[34] that this can not occur in the presence of full amounts of iodin.

OBSERVATIONS ON THE IODIN CONTENT OF THE THYROID GLANDS OF OTHER ANIMALS.

Since it has been shown by many observers that in goitrous districts all mammals are, to a greater or lesser extent, prone to thyroid changes, it is proper that tables be made both of their anatomic changes and their iodin contents in order that the physiologic importance of iodin can be estimated. Though we have merely begun this task, the results may be hinted at and suggestions made.

Human Examinations. — Of two cases anatomically classified as "colloid X early," glandular hyperplasia, one was clinically normal and contained 0.208 mg. of iodin per gm. of fresh thyroid, while the other case was an old colloid goiter on which was engrafted an early hyperplasia. This case was clinically regarded as early exophthalmic goiter engrafted on an old colloid goiter. The gland contained 0.162 mg. iodin per gm. of fresh thyroid. In these two cases the anatomic changes and their iodin contents agree and are similar to the findings in dogs. Of four other cases clinically regarded as exophthalmic goiter (mild cases), who had, however, been treated with iodin, all were anatomically pure colloid goiters and showed iodin contents of 0.304, 0.633, 0.707, 0.883 mg. respectively, thus agreeing with the observations of the others on the rapid storage of iodin, and also agreeing with our anatomic observations indicating that there is a return to the colloid type on the admin-

34. Cleveland Med. Jour., February, 1908, vii, 105.

istration of continued minute doses of iodin even in the exophthalmic hyperplasias.

Sheep.—An hyperplasia which had undergone spontaneous reversion contained 0.455 mg. iodin per gm. of fresh thyroid, while four marked hyperplasias showed in two cases no estimatable amount, and in the other two iodin contents of 0.014 and 0.019 mg. per gm. of fresh thyroid respectively. These observations suggest that here, too, the iodin was proportional to the colloid, and inversely proportional to the degree of glandular hyperplasia.

Horses.—Two thyroids in the anatomic stage of moderate glandular hyperplasia showed 0.054 and 0.091 mg. iodin per gm. of fresh thyroid respectively.

Calves.—A large parenchymatous goiter in the stage of marked glandular hyperplasia contained 0.042 mg. iodin per gm. of fresh thyroid.

Cat and Bear.—On examination a histologically normal cat's thyroid showed 0.154 mg. of iodin per gm. of fresh gland, while the thyroid from a young bear in the stage of early hyperplasia contained 0.081 mg. iodin per mg. of fresh gland.

These observations, though still scanty, suggest that other animals in general obey laws similar to those found for the dog, and also, as pointed out by Baumann, that different animal species have normally different iodin contents—the carnivora having considerably less than the herbivora.

In reviewing the investigations of Baumann, Roos, Oswald and Weiss we find that direct comparisons with our work are impossible, because the methods of iodin determinations differed in some details, but especially because no histologic classification was used, which renders it impossible to estimate what relation iodin bore to the gland structure. However, a summary of their results may be of interest. Baumann and Roos,[35] working with sheep's thyroids, found that their iodin content varied with the regions from which the glands were taken, being least in the goitrous regions, e. g., Frieburg glands contained 1.06 mg. per gm. of dried gland; Elberfelt glands contained 3.40 mg. per gm. of dried gland; Paris glands contained 1.70 mg. per gm. of dried gland.

Baumann,[36] working with human glands, found the same regional differences in the iodin content of normal adult glands as was noted for sheep, e. g., Hamburg glands contained 0.83 mg. iodin per gm. of dried

35. Ztschr. f. physiol. Chem., 1895-6, xxi, 480.
36. Ztschr. f. physiol. Chem., 1896-7, xxii, 1.

gland; Berlin glands contained 0.90 mg. iodin per gm. of dried gland; Freiburg glands contained 0.33 mg. iodin per gm. of dried gland.

He also found that the same differences held for infants and children. In goiters obtained in Freiburg he found 0.09 mg. per gm. of dried gland to be the average iodin content. From these observations he concluded that thyroids from animals in goitrous districts have normally less iodin than glands from non-goitrous regions, and that in uncomplicated goiters it was exceedingly low.

Oswald,[37] working with normal (?) glands from the several provinces of Switzerland, found that the glands contained as much iodin (0.916 mg. per gm. of dried gland) as normal glands from non-goitrous districts, but that goiters (glands weighing 95 gms. or more) contained, on the average, about half as much iodin per gm. of dried thyroid. Weiss[38] found the average iodin content in human glands to be 0.56 mg. per gm. of dried glands for Silesia.

OBSERVATIONS ON THE IODIN CONTENT OF OTHER TISSUES.

From the examination of the livers and muscles in three cases, in two of which iodin had been administered, the thyroids showing a high iodin content, only the slightest possible trace of iodin was present in two pieces of muscle, and none in the livers. We think these findings suggest that the amount of iodin normally present in tissues other than the thyroid is a negligible quantity.

Recently Loeb,[39] from a rather extensive study of the tissues (rabbit) after the administration of known quantities of potassium iodid, arrived at similar conclusions., viz., that after ninety-six hours following the administration of KI to rabbits, mere traces only could be detected in the lungs, kidneys, skin and blood.

If Justus'[40] contention that iodin is present in all cell nuclei be true, it must be so small in amount that it is not determinable by the methods adopted by us. In one case in which two accessory thyroids were examined, an animal to which small amounts of iodin had been administered, a rather high iodin content (0.1 mg.) was found. This is to be expected, since the accessory thyroids (examined histologically in a large number of cases) show similar anatomic changes to those occurring in the main lobes.

The thymus from the same case also showed a relatively large amount

37. Ztschr. f. physiol. Chem., 1897, xxiii, 265.
38. Münch. med. Wchnschr., 1897, xliv, 6.
39. Arch. f. exp. Path. u. Pharmakol., lvi, 320.
40. Virchow's Archiv., Bd. clxx, S. 501.

(0.046 mg.) of iodin. Gross inspection of the gland as well as study of the portions taken for microscopic examination, failed to reveal accessory thyroids. This is at variance with Cunningham and Mendel,[41] who claim that when iodin is present in the thymus it is probably due to the presence of accessory thyroids. In fairness, however, it may be stated here that there is another possible explanation of the presence of iodin in the dog's thymus, viz., that over 20 per cent. of the dogs show persistent cystic dilatation of the embryonal thymic ducts (first described by Remak), and that the iodin present may be the result of excretion into these spaces rather than evidence of its existence in the true functional thymic tissue. Baumann[42] was the first to point out that the thymus contained iodin. He also showed that by feeding dogs with fresh thymus, as compared with feeding with fresh muscle,[43] the iodin content could be raised; this could also be done in the case of thyroid glands by feeding with dog biscuit or sea fish. That the thymus is in many ways related to the thyroid in function has been pointed out by many observers from several points of view. For example, Mikulicz[44] and others have noted the mildly beneficial effects of feeding thymus in goiter; it has also been pointed out that in parenchymatous goiter and in cretins the thymus is generally persistent or even enlarged.[45]

Whether iodin is in any way related to the therapeutic value of thymus or its associated persistence in cretinoid states is a subject on which one of us (Marine) is now collecting data.

OBSERVATIONS ON COMMERCIAL DESICCATED THYROID.

As pointed out in a previous paper by one of us, a large proportion of the sheep in certain districts have enlarged (hyperplastic) thyroids, and it occurred to the writer to visit one of the larger commercial laboratories where desiccated thyroid is prepared to see what kind of glands were being used. It was found that they were using hyperplastic as well as normal glands in preparing their medicinal products. In the light of the findings of Baumann, Oswald, Roos and the authors, viz.: (1) that the iodin content, in general, varies with the amount of colloid; (2) that in general the colloid varies inversely with the degree of glandular hyperplasia; (3) that the iodin content varies inversely with the degree of glandular hyperplasia, and (4) that the physiologic activity

41. Am. Jour. Physiol., 1900, iii, 285.
42. München. med. Wchnschr., 1896, liii, 311.
43. Ztschr. f. physiol. Chem., 1896-7, xxii, 14.
44. Berl. klin. Wchnschr., 1895, xxxii, S. 342.
45. Marie, Gaz. d. hôp., 1893, p. 202; also Hektoen, Internat. Med. Mag., 1896, iv, 584.

of thyreoglobulin varies directly with the amount of iodin in organic combination, it becomes necessary to determine the iodin content of the various thyroid preparations. Accordingly, one of the firms prepared for us samples made from what they considered (1) normal glands (gross appearance), and (2) hyperplastic glands (gross appearance). We add the iodin contents of these specimens, and for comparison the iodin contents of a sample obtained in the market, together with a determination made from histologically normal glands.

Specimen "C" from hyperplastic glands,, 0.292 mg. iodin per gm. of dried gland; specimen "D" from normal glands, 1.092 mg. iodin per gm. of dried gland; specimen "E" obtained in the market, 1.538 mg. iodin per gm. of dried gland; specimen "F" histologically normal glands, 1.769 mg. iodin per gm. of dried gland.

These four analyses indicate that the preparation made from the hyperplasias contains only one-sixth the amount of iodin present in the histologically normal glands, while a sample obtained in the market contained a third more iodin than the sample made from supposedly normal glands. There being no reason to suppose that iodin was added, no attempt was made to determine whether there was any free iodin. Since the manufacturing firms use both the hyperplastic and the normal glands in the preparation of their therapeutic products, it follows that different samples must contain varying amounts of iodin. Aufrecht examined thyroid tablets made from hog's glands obtained from different localities and found great variations in the iodin content.

Experiments with feeding specimens "C" and "D" desiccated thyroid were undertaken and, although accidentally incomplete, are suggestive enough to be mentioned here. Two dogs (A-107 and A-108) of the same age, breed and weight were fed on equal amounts (11 gms.) of specimens "C" and "D" respectively in eighteen days. The dog fed with specimen "C" (hyperplasia) did not lose weight, and the amount of iodin per gm. of fresh thyroid was 0.173 mg. (gland taken twenty-one days after last dose of substance); while the other dog (A-108) fed on specimen "D" (normal glands) lost 454 gms. in weight in eighteen days of feeding; the amount of iodin per gm. of fresh thyroid was 0.439 mg. (gland taken twenty-one days after last dose). This suggests, as Oswald and Roos have pointed out, that the activity of the thyroid substance varies with the amount of iodin in organic combination; but it is not in contradiction with the possibility and, indeed, the probability,

as mentioned by Hunt[46] and one of us,[1] that the substance, in the absence of iodin, may have other activities.

GENERAL DISCUSSION.

The importance of a histologic basis of classification in goiter can not be overestimated. Virchow, as early as 1863, pointed out the great confusion which then existed in the classification of thyroid diseases and also the dangers to which it was leading, viz., the interference with the advance in our knowledge of goiter both anatomically and clinically. In the first place, the classification must be rigid, and second, it must be dependent on the three natural anatomic divisions., viz., (1) normal glands; (2) colloid glands; (3) hyperplasia. In an organ whose structure is so manifestly affected by a variety of systemic conditions it is clearly impossible to make use of any other factor than anatomy as a point of departure. Little need be added relative to the anatomic descriptions of the groupings already given. It is to be regretted that more normal glands were not found, though it seems unlikely that iodin determinations in a larger number of normal glands would have materially changed the relative importance, and it is this feature, rather than the actual figures, that we consider important.

Comparison of the iodin determinations with the corresponding histologic groupings, as shown in Tables 2, 3, 4 and 5, permit the deduction of several important general conclusions which bring the relationship of iodin to the structure of the thyroid closer than has heretofore been suspected. Some of these relationships may be again referred to, for the sake of emphasis: the percentage of iodin in general varies with the amount of stainable colloid, as was first pointed out by Oswald. To this rule there is an apparent contradiction in the normal and colloid gland, but this can be explained by the fact that there are quite wide limits in the percentage of iodin above the amount necessary for the maintenance of normal structure, and this reserve iodin, so to speak, produces no detectable microscopical changes. It also appears to be a general law that the colloid material in general varies inversely with the degree of glandular hyperplasia, and third, it is shown that the iodin content varies inversely with the degree of glandular hyperplasia.

In the analyses of the individual members of the separate groups there are many apparent exceptions also to the last two general laws mentioned, and these exceptions rather suggest that while iodin is the main object and factor governing thyroid activity, there are other con-

46. The Journal A. M. A., 1907, p. 1323.

ditions as well which influence the gland structure. This, indeed, would be probable if goiter is considered as a manifestation of a nutritional disturbance.

As pointed out above, there are wide variations between the amount of iodin necessary to maintain normal structure and the largest amount which can be held in organic combination by the colloid (thyreoglobulin), and within these limits the microscopic changes in the structure are too slight to be estimated accurately, though there must be wide differences in structure that are not to be detected by present microscopic methods. In view of this factor of error in normal and colloid glands, more importance should be attached to the early hyperplasias and to the minimal amount of iodin necessary to start hyperplastic changes. This minimal amount is found to be quite constant, as shown in Table 5, being 0.173 mg. iodin per gm. of fresh thyroid for the "normal X early" glands, 0.217 mg. for the "colloid X early," and 0.139 mg. for the pure early glands.

Passing to the hyperplasias, here, too, we find a difficult factor to determine by histologic examination for the reason that microscopically it is often difficult to decide whether a gland is reverting from a more marked hyperplasia or is undergoing secondary hyperplasia; in the former case the iodin content would be higher than in the latter. So that, in view of these known difficulties, it is probable that future workers, while laying most stress on the anatomic structure, will have to take cognizance of the iodin content as well, in order to group the glands accurately and to estimate their functional activity. Nevertheless, it is clear that these apparent exceptions do not materially affect the importance established for iodin in its relation to thyroid structure and gland activity, and, besides, it is probable that all these exceptions could be rationally explained if the complicating factors acquired during the life of the animal were known.

A point worthy of special notice is that the iodin contents of the glands included under double references, as shown in Table 5, fall between the iodin contents of the groups to which they were anatomically referred, though in making up the original tables they were not considered at all, and the results could not in any way be influenced by ideas gained from the original (pure) groups. This additional grouping amounts, in effect, to doubling the evidence in proof of the three laws above mentioned, and also indicates that the hyperplasias, both histologically and chemically, resist arbitrary grouping, but represent a continuous gradation from the normal glands to the marked hyperplasias.

In a previous paper[1] it has been pointed out that the thyroid is not the only tissue affected in the production of glandular hyperplasia (parenchymatous goiter). As has been pointed out by many observers, the lymphoid tissues (spleen, thymus and lymph glands), together with the bone marrow, show quite constant lesions that must be considered integral parts of the general disturbance, and when one recalls the experimental biologic data already mentioned in the introduction it appears highly probable that in the natural occurrence of goiter, as in its artificial production, the underlying process is essentially a "compensatory reaction to some deficiency" rather than that it is a spontaneous thyroid affection. This is a view quite opposite to that held by many students of exophthalmic goiter. That iodin is the most important single factor in this disturbance is further suggested by the fact that the ability of the thyroid to store iodin depends on the degree of glandular hyperplasia rather than on the form or mode of its administration, as shown in Table 9, and also that there is an economy and storage of iodin (somewhat comparable to that of iron), as shown by the fact that there are wide limits in the iodin content between the amount necessary to maintain normal structure and the highest amount of iodin which can be stored in the gland.

In view of the evidence thus far deduced, we believe that goiter must be classed with the nutritional disturbances (diseases) and that as such it will take its place in nosology along with chlorosis, rachitis, status lymphatics, osteomalacia, (?) etc. That there are other factors than iodin influencing the thyroid changes seems to be proved, but there is no experimental evidence that a specific infecting agent as such is one of these factors.

The mode of action of iodin is probably similar to that of iron, calcium and other normal metallic constituents of the body in their relations to normal and disturbed metabolism, viz., that as in the case of iron, calcium, etc., so with iodin, (1) the body practices an economy in its use; (2) there is a normal minimal amount necessary for the body needs, and a variable reserve quantity, and (3) that a deficiency of either element induces anatomical changes in the tissues directly concerned with their storage and elaboration.

Finally, the treatment of simple hyperplasias has close analogies with the treatment of chlorosis, particularly in that the form of iron or iodin administered is not all important, since the ability to take up these metals depends rather on the degree of tissue reaction. And this fact suggests a point in the treatment of symptomatic thyroid hyperplasias

that may have considerable practical importance, viz., that the physiologic dose of iodin should be inverse to the degree of glandular hyperplasia.

The reversion to a colloid type, which occurs coincidently with the administration of iodin in a hyperplasia, is the histologic evidence of a cure and not of a degenerative process in the usual interpretation of that term. Colloid glands (goiters) are the nearest normal condition to which the hyperplasias can return, and not only is this true of their gross and histologic appearance, but also of their chemical (iodin) and clinical bearing as well.

The problem of commercial desiccated thyroid is an important one for the pharmacologist and clinician alike, as has already been shown both on the bases of anatomic structure of the thyroids used and on its physiologic activity. Some standard, either chemical or physiologic, is needed, even if in the absence of organically combined iodin the substance is inert. This, however, can not be granted at present, as there is some clinical evidence that these hyperplastic glands during life have a greatly altered secretion which exerts a toxic action on the organism. Whether this activity may be destroyed in the preparation of desiccated thyroid is a very important problem for clinical experiment, particularly when desiccated thyroid is so extensively used in the treatment of thyroid changes histologically similar to those existing in the glands from which the preparations are made.

PROBLEMS OF HUMAN GOITER.

In view of the relations that have been shown to exist between the amount of iodin and the histologic structure of the thyroid in the dog, and in view of the facts thus far collected as to the incidence, distribution, nature and treatment of goitrous conditions in all animals, it seems necessary that similar work be carried out in man, both in goitrous and non-goitrous districts. The essential problems for solution must be the same in man as in animals, and are modified only by man's position in the biologic scale and by the vast complex of acquired conditions—accidental or intentional (social complex, food, migration, locality, medication, etc.).

With fairly definite anatomic criteria established in animals, it seems possible to group and study the human forms. This work is now in progress. As is to be expected, great irregularities are met with on account of complicating factors which are almost always present and make interpretation of the observations difficult. However, the general conditions so far are found to obey the same general laws that were

TABLE 3 To Accompany Article by David Marine M.D., and W. W. Williams M.D. on Relation of Iodin to the Structure of the Thyroid Gland

Number	Sex	Weight and Breed and Age	Microscopic: Nuclear Colloid	Nuclear Early	Glandular Hyperplasia Moderate	Glandular Hyperplasia Marked	Chemic: Weight of the gland	Weight of the thyroid	Percentage of water	Iodin per cent	Iodin per kilo fresh tissue	Iodin per gm. fresh tissue	Iodin per gm. fresh tissue dried	Remarks
A 1	M	7.60 Fox Young					1.1	0.1		1.1	0.01	0.5	0.07	
A 2	M	9.07 Short Medium					1.1	0.1		1.1	0.01	0.5	0.07	
A 3	M	7.26 Fox Medium					1.1	0.1		1.1	0.01	0.5	0.07	
A 4	M	8.2 Fox Medium					1.1	0.1		1.1	0.01	0.5	0.07	
A 5	M	8.64 Fox Medium					1.1	0.1		1.1	0.01	0.5	0.07	
A 6	M	8.64 Fox Medium					1.1	0.1		1.1	0.01	0.5	0.07	
A 7	M	8.64 Fox Medium					1.1	0.1		1.1	0.01	0.5	0.07	
A

found in the dog. The problem of human goiter at the present time is practically one of a study of toxic (exophthalmic) goiter, since the simpler forms are easily controlled.

The investigations in the past have been largely concerned with the elaborations of methods of treatment or a discussion of the various symptoms and signs. The underlying factors of most of these signs and symptoms are either unknown to us or are common to other nervous and mental affections as well. Further advance in our knowledge as to the nature of the process from the purely clinical side is, therefore, scarcely to be expected. The first step in undertaking a systematic study of exophthalmic goiter, it seems, should be that of providing a definite, strict anatomic classification, checked in every instance by chemical and physiologic examinations of the thyroid, after which we must seek to bring the clinical observations into relation with these findings.[47]

CONCLUSIONS.

1. Iodin is necessary for normal thyroid activity.

2. Iodin is the main index to the physiologic value of the thyroid secretion (thyreoglobulin) to the body.

3. The percentage of iodin varies with the amount of colloid in the several degrees of hyperplasia; the colloid varies inversely with the degree of thyroid hyperplasia; the iodin varies inversely with the degree of thyroid hyperplasia.

4. The colloid gland (goiter) is the quiescent or normal state of a gland which has previously been a glandular hyperplasia and obeys all the biologic laws of a normal gland.

5. In the classification of thyroid changes (excluding infections and neoplasms) four major groups must be recognized, viz.: (1) normal glands, (2) colloid glands, (3) hyperplastic glands (all grades), (4) complications engrafted on any of the three preceding groups.

6. The ability of the thyroid glands to store iodin depends on the degree of glandular hyperplasia rather than on the form or mode of administration of iodin.

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47. It is impossible to ascertain the anatomical condition of the gland except by histologic examinations, and in the determination of normal glands this is doubly important. It should be pointed out that in a scientific investigation there is no "normal gland for a district," as was stated by Oswald. He considered a gland non-goitrous in Switzerland if it weighed less than 95 gms. (normal adult sea coast glands average 30 to 40 gms.). Our standard of normal is borrowed from the sea coast type of gland and in goitrous districts this standard is rarely attained.

7. The optimum therapeutic effects of iodin on the thyroid gland are only obtained with doses vastly smaller than those usually employed.

8. The percentage of iodin which a thyroid may contain is variable, but the minimal percentage necessary for the maintenance of normal gland structure is quite constant.

9. Commercially desiccated thyroid has a very inconstant iodin content and, therefore, has a variable physiologic activity and therapeutic value.

10. The problem of human goiter, both anatomically and chemically, should be investigated along lines similar to those adopted for the dog.

[NOTE.—We wish to thank Prof. Torald Sollmann for his careful criticisms and suggestions; Prof. W. T. Howard for the privilege of his autopsy material, and Prof. G. W. Crile for the free use of material from his surgical clinic. We are also greatly indebted to Drs. C. H. Lenhart and M. B. Bonta for their skilled assistance in the operations.]

LYMPHANGITIS AND PERILYMPHANGITIS OF THE LIVER IN THEIR RELATIONS TO THE INFLAMMATIONS OF THE ORGAN.

HORST OERTEL.

NEW YORK CITY.

[From the Russell Sage Institute of Pathology, New York City Hospital.]

The importance of the lymphatics in the inflammatory lesions of parenchymatous organs is well recognized. They are not only frequent carriers of infection, but in the absorption of inflammatory products their condition becomes a paramount issue in the ultimate fate of the organs.

We know that in pneumonia, for instance, the state of the lymphatics determines the resorption of the exudate and, therefore, the termination of the whole disease. Obliteration of the lymphatics by an acute lymphangitis and perilymphangitis retards the resolution until these paths have been cleared, and, if this does not take place, aids the termination into gangrene or abscess. Again, permanent destruction of lymphatics, as in chronic essential emphysema and in senile atrophy of the lung, are grave factors against rapid removal of inflammatory exudates and are almost entirely responsible for the long-continued, irregular course often taken by inflammations of the lungs, as well as for their frequent fatal termination in persons who already have the above-mentioned diseases. The same considerations apply with equal force to other parenchymatous organs. In the liver, however, their importance as carriers of infection and as determining factors in the course and termination of inflammatory lesions has been less emphasized than in connection with other organs—certainly much less emphasized than the rôle of the bile ducts and blood vessels, especially the vessels of the portal circulation. It is erroneous, however, to believe that the lymphatics of the liver may be disregarded or viewed as of secondary importance, for they present very definite and important changes in the various inflammatory conditions of this organ.

I shall confine myself in this article to the condition of the lymphatics in the "acute," "subacute," and "chronic" inflammations. For their study we have had unusual opportunity in this laboratory during the last few years, and some interesting cases have already been described

in their main features.[1] These cases, of which I need not repeat the details here, as well as some other typical "acute" inflammations occurring in connection with general infections, "chronic" forms, given in cirrhosis of the liver, and finally some found in acute exacerbations of "chronic" cirrhosis, furnish the basis of these observations. I shall disregard for the present specific affections, like the infective granulomata, in their relation to the lymphatics of the liver, leaving them for future consideration.

The liver, as is well known, is supplied by a rich network of lymphatics which is derived from two sources. The first and most important set, as far as the parenchyma is concerned, is that which accompanies the portal vessels, the bile ducts, the hepatic artery and the hepatic vein. The second and superficial set forms an extensive network within the capsule, and communicates with the first by means of channels through Glisson's capsule. Whether lymph channels occur within the acini is still a disputed question. From the pathological standpoint both of these lymphatic systems of the liver appear to have at least some degree of independence, for severe affections may remain confined to one without involving the other. Perhaps the best illustrations of this distinction are found in cases of Curschmann's so-called "sugar-glazed" liver, which is a hyperplastic deforming perihepatitis, or in cases of Pick's pseudocirrhosis, or in cases of polyserositis or hyaloserositis. Common to all of these is an enormous productive inflammatory thickening of the serous coat of the liver with extensive involvement of the superficial lymphatics only. On the other hand, in my experience, it is very rare to find extensive lymphatic involvement of the superficial or capsular set in inflammations of the liver itself, but these show marked disease of all internal lymphatics of a type which I shall presently describe. The only exceptions are cases in which a complicating peritonitis affects the lymphatics of the capsule.

It follows, therefore, that as far as the fate of the liver itself is concerned the affections of the internal lymphatics are the paramount issue, and I shall confine myself to them.

First, then, with regard to the so-called acute and subacute (better, exudative and productive) inflammations.[2] I shall consider these together on account of their close relationship and the similarity of the affections

1. Symmers, D.: Am. Jour. Med. Sci., 1908, cxxxv, No. 2.

2. I use these terms in Cohnheim's and Orth's sense: Cohnheim, Vorlesungen über allgemeine Pathologie, 1877, p. 281; also Orth's Lehrbuch der speziellen pathologischen Anatomie, Berlin, 1887, Preface p. 4. Others speak of proliferative instead of productive inflammations (Ziegler).

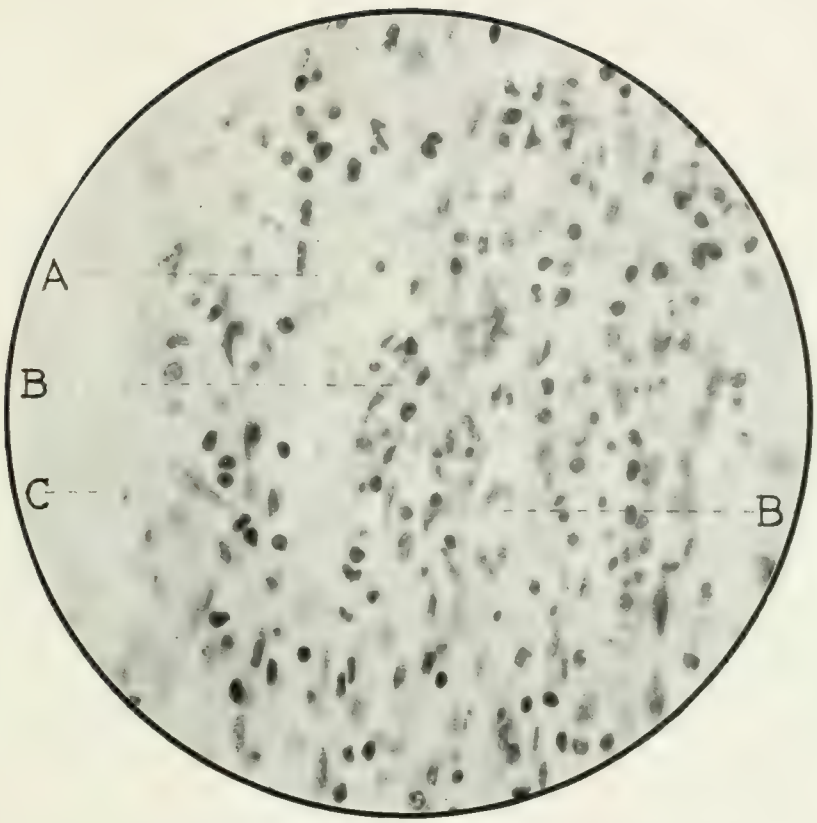


Fig. 1. -Exudative lymphangitis and perilymphangitis of a portal space (exudative interstitial inflammation of the liver); acute diffuse hepatitis. *a.* Blood vessel. *b.* Dilated lymph spaces filled with serum, leucocytes, mononuclear cells (desquamated endothelial cells.) *c.* Adjoining cloudy liver-cells. Zeiss: Oil immersion 2 mm. Ocular 4 mm.

of the lymphatics in the two. Changes occur here partly within and partly without the lymphatic channels of the interstitial tissue between the lobules. The lymphatics are generally dilated with stagnant lymph; they are filled with coagulum and large and small mononuclear cells of the lymphoid type, desquamated endothelial cells and polymorphonuclear leucocytes (Fig. 1). In some cases this condition is well marked in the initial stages of the lesion, and it may form the central feature of the inflammatory affection of the interlobular septa—an acute lymphangitis. It may be immediately recognized by the regular streaky infiltrations of the interstices following exactly the line of the lymphatic courses. More frequently an involvement of the surrounding structures follow. As a result of the blocking of the lymph stream, the perilymphatic tissue becomes edematous (Fig. 1). This leads primarily to widening of the tissue spaces around the lymphatics, and the cellular infiltration with lymphocytic cells and polymorphonuclear leucocytes extends now to these structures. It may remain confined to the tissue spaces in the immediate neighborhood of the lymphatics, or it may, especially in advancing lesions, infiltrate more or less diffusely all the interstitial tissue. In other words, the lymphangitis becomes associated with a perilymphangitis.

The anatomic distribution of the lymphatic channels in the liver, which is mainly along the paths and in the sheaths of the vessels and the bile ducts, necessarily implicates them in all affections which take their origin from or around these latter structures. Even in these cases the lymphatic involvement, although secondary, becomes of great importance in the dissemination of the inflammation, for the lymphatics will not remain locally affected, but will carry infection to other distant parts of the liver substance. This factor reserves for the lymphatics a certain independence even in those cases in which they have become secondarily affected as the result of primary biliary and vascular infections. In other words, it is not necessary that the lymphangitis and perilymphangitis progress *pari passu* with the inflammation of the ducts and the vessels; nor does the severity of the lymphatic affection stand in a constant definite ratio to that of the bile ducts or the blood vessels. The active inflammatory process of the lymphatics necessarily modifies the extension and severity of the affection in each case. It has already been mentioned that we must attach to them an important rôle in the dissemination of a lesion. On the other hand, early and complete plugging of the lymphatics would form an aid for the localization of an infection. Further—and herein lies a very essential feature—

all resorption of inflammatory products must be delayed or entirely suspended in areas thus affected, as long as the lymphatic circulation is interfered with by blocking of lymphatic and perilymphatic spaces. Whether, then, the liver is restored to its former integrity, or whether inflammatory changes will continue to spread or become permanent, depends largely on the status of the lymphatics. In order to have restitution to integrity it becomes necessary here, as in other parenchymatous organs, to have clear lymphatics. Although a morphological control is not possible, there can be no doubt that this occurs in a large number of cases in which the liver is "acutely" affected, but in a number of others, and they are the ones going on to productive inflammation and to true cirrhosis, restitution and clearing of lymphatics does not take place, but they show changes which, while they are not of the same exudative type as those previously described, are just as definite and important in their relation to the ultimate outcome of the disease. In these delayed cases of restitution, in which the progress of the disease is manifest, the changes in the lymphatics are especially characterized by endothelial proliferation of their walls. This endothelial proliferation leads not only to thickening of the wall, but gradually to obstruction of the lumen of these vessels, for endothelial cells gradually accumulate within the channel and sooner or later the lumen is entirely closed. This obliteration is considerably aided by free fibroblastic proliferation from without, which further tends to compress these lymphatic channels. The occlusion of these interstitial lymphatics goes, then, hand in hand with the formation of maturing fibrous connective tissue (Fig. 2). An interesting feature in this connection is the formation of new lymph channels along with new capillaries. Undoubtedly they serve here the same compensatory purpose that the newly-formed capillaries do, but, unfortunately, a large number of these newly-formed lymphatics suffer the same fate that many of the newly-formed blood vessels do; they are gradually squeezed out of existence in the maturing and growth of the increasing connective tissue.

These more slowly progressing changes in the lymphatics are no less important in the productive inflammations of the liver than they are in the exudative conditions. In the first place, it seems evident that a large number of cases that begin as exudative interstitial hepatitis are considerably aided in assuming an additional productive character or in becoming purely productive, by obliteration of the lymphatics; for this must necessarily interfere with the proper lymph stream and thereby with the proper nutriment of the parts, and thus with the resorption of the inflammatory products. As a consequence the parenchyma suffers severely

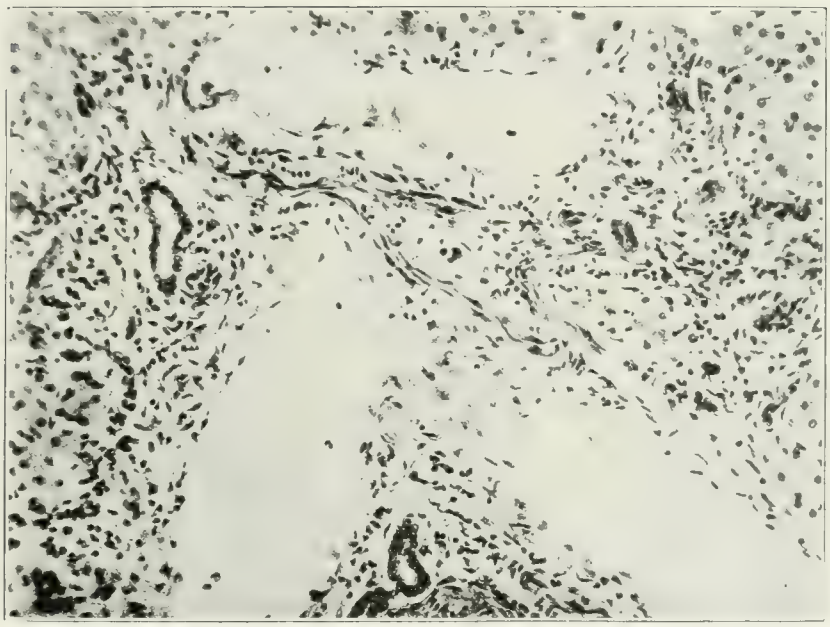


Fig. 2.—Recent productive hepatitis with productive lymphangitis around the vessels. Zeiss: Objective 16 mm. Ocular 12 mm.

and progressively; it degenerates and wastes, and favorable conditions for the production of a cirrhosis are given. Then, as fibroblastic proliferation progresses from outside, and their own endothelial proliferation from the inside, the interference with these functions becomes greater and greater, and as a result the injury to the parenchyma increases correspondingly. While an attempt is made to counteract this by the formation of new lymph channels, along with newly-formed capillaries, this is in all probability not sufficient, and in the majority of cases these newly-formed lymphatics are not even permanent, but are lost by the slow but constantly progressing inflammatory production of new tissue.

To recapitulate: Inflammatory conditions of the liver are associated with an exudative lymphangitis and perilymphangitis. If, in the subsidence of the inflammatory irritation, the lymphatics are cleared and nothing stands in the way of resorption of inflammatory irritants and products, a change to the normal may take place. If, on the other hand, the lymphatics are not cleared, they form an essential factor in the establishment of a progressing inflammation in the form of an exudative and productive or pure "cirrhosis" of the liver.

Viewed in this light, some forms of cirrhosis of the liver appear primarily only as an inflammation of the portal spaces, which, on account of unfavorable conditions, particularly for resorption, and a consequent interference with nutrition, lead to degeneration and loss of parenchyma. This is necessarily followed by a fibroblastic proliferation which replaces wasted areas. Maturing in the more slowly progressing forms, this inflammation is apt to undergo frequent exudative exacerbations. These will involve the lymphatics in exactly the same fashion as in a previously normal liver, and they will, therefore, become diffuse and lead to further injury of the parenchyma. In a liver already much changed such acute exacerbations are apt to lead to speedy exitus.

I said before that I believe that *some* forms of cirrhosis have this pathogenesis, for I do not want to imply that *all* are the result of such changes. On the contrary, there are undoubtedly forms which commence with marked injury to the parenchyma as well, and in those a hyperplastic proliferation of the connective tissue seems to follow more closely in the path of this primary, parenchymatous destruction. Histologically these two forms present distinctions. The first group includes all those cases in which, especially early in the disease, the parenchyma appears relatively well preserved, and the inflammation and formation of fibrous tissue appear almost out of proportion to the degeneration of the parenchyma. In the second group, however, are all cases in which the

parenchyma shows, even early, an extensive parenchymatous and fatty degeneration, bile imbibition and various forms of cytolysis. There the inflammatory interstitial changes are irregular, not so distinctly periinsular, but diffuse, plainly intralobular, and this diffuse infiltration which allows free invasion of inflammatory products is made possible by primary, severe involvement of the liver cells themselves. In this connection I should draw attention to the fact that the classification of the various forms of cirrhosis of the liver, which has been especially attempted by the French and to a large extent followed in this country, appears perfectly impossible from an anatomical standpoint, for these forms appear of such close relation, and merge so freely into one another, that it seems unjustifiable to attempt to hold the cirrhoses too rigidly into definite classes. We should rather view them as of a uniform nature modified only by the number and quality of surrounding conditions in each case.

One great mistake which has frequently been made, even by pathologic anatomists, the distinction of two great groups of cirrhoses, namely, the so-called interlobular and intralobular forms, is certainly based on false interpretation. As a matter of fact, all forms of cirrhosis are intralobular, for there is no cirrhosis, not even the old typical Laennec's cirrhosis, which is not intralobular. The pictures in this form of cirrhosis which have been wrongly interpreted as being the result of a distinctly interlobular formation of fibrous tissue do not in reality present such a process. They are produced only by the slow advance of bands of mature fibrous tissue into the liver parenchyma whereby groups of liver cells are cut off from their surroundings and appear in the form of islands, which have been wrongly interpreted by some writers as representing always individual lobules or groups of lobules. More correctly, these islands are frequently produced by cutting off various portions of a number of acini by a distinctly intraacinar growth of fibrous connective tissue. The distinction between this form of cirrhosis, then, and the so-called intralobular form lies not in a characteristic typical distribution of connective tissue, for in both of them this is an intralobular growth; it depends rather on the surrounding conditions of each case, which in the one limit its growth by better parenchymatous preservation, while in the other the growth is diffuse from the start on account of the greater injury to the parenchyma. Whatever other features may become associated with any case, they are not characteristic of particular groups of cirrhotic processes, but are determined by the accompanying conditions of each

case. These are mainly angiocholitis, bile duct formation, and lesions leading to stasis and ascites.

The terms interlobular and intralobular, as distinctive features of types of cirrhosis, are, therefore, false, and for descriptive purpose the terms periinsular and diffuse would be decidedly preferable. Undoubtedly combinations of the two classes are not infrequent, and this is especially well shown in cases of old periinsular cirrhosis which undergo exudative exacerbation. Here observation proves, in addition to the old mature periinsular cirrhosis, the recent more diffuse spread of inflammatory products. This may be interpreted as being due to the result of the previous interstitial inflammation, as I outlined it above, which has injured the remaining cells sufficiently to allow a free entrance of the inflammation into the remaining islands whenever an exudative exacerbation occurs. All "acute" exacerbations, then, of chronic cirrhosis lead to the so-called "mixed forms," that is, to a very diffuse distribution of the newly-formed fibrous tissue, which must, necessarily, be associated with more complicating secondary results than the limited old form upon which it was implanted.

In the discussion of the pathogenesis of cirrhosis of the liver we have, as is well known, two opposing views. Some regard it as a primary inflammatory interstitial growth and consider that the loss of parenchyma is dependent upon it. Others, however, deny the possibility of this extensive connective tissue proliferation without previous injury and waste of parenchyma, and believe the fibrous tissue growth to be an entirely compensatory process.

I am inclined to take a stand somewhat between these two, and more or less reconciling them, inasmuch as I believe that the origin of the true cirrhosis of the liver must be looked for in the interstitial inflammation; and that either this may be associated from the start with a severe injury to the parenchyma, which involves diffuse, irregular, fibrous tissue growth throughout the liver, or the parenchyma may become involved much more gradually and largely secondarily on account of the previously outlined progressive features of this interstitial inflammation. In such instances the parenchyma wastes more slowly, as a consequence of which the connective tissue grows in a more or less periinsular fashion. While, then, the origin of the cirrhosis is primarily an interstitial inflammation, the cirrhotic process proper is eminently a compensatory process which depends upon extensive or more slowly progressing, irregular destruction of the parenchyma. A combination of the two types is possible and not at all infrequent.

I can not leave this subject without a few remarks about the so-called hypertrophic cirrhosis, or Hanot's type. A proper discussion of this lesion, which, as some insist, is a distinct anatomic and clinical entity, is made extremely difficult by the conflicting testimony of its own advocates. The characteristic features of this so-called entity have been variously described, either more grossly as hypertrophy or, better, as enlargement of the liver with a smooth surface and jaundice, a large spleen and no ascites, or, microscopically, as an exclusively intralobular formation of thin connective tissue fibrils between individual liver cells around the bile ducts, with cholangitis and new bile-duct formation, and finally preservation of liver cells. Some would regard one or the other, or several of these, as the distinguishing features, and dispute the others, and few agree even with Hanot himself about the character of the process. This disagreement of authors has gone so far that a few now proclaim that the intralobular formation of delicate fibrous connective tissue, which is by every one practically admitted to occur in this disease, was not a feature of the original findings of Hanot.

Cases of cirrhoses with more or less smooth enlargement of the organ were observed from time to time before Hanot, and Hanot himself has drawn attention to these in several of his writings on the subject. Notable among them is the one by Hayem,[3] 1874. But it was Hanot who primarily insisted on the establishment of a special type on the basis of some cases which he had collected and presented in 1876. He founded his description on a number of partly anatomic and partly clinical peculiarities, which, in order to avoid confusion, I will quote in his own words found on page 89 from the original publication:[4]

It is, therefore, clear that Hanot recognized from the start an intralobular as well as an interlobular connective tissue growth without contraction, and also a catarrhal condition of the bile ducts with their abnormal development. His clinical characteristics need not detain us here, but it is interesting to know that he distinctly contrasted the pathogenesis of atrophic and hypertrophic cirrhosis, inasmuch as he was led to believe

3. Hayem: Contribution a l'etude de l'hepatite interstitielle chronique avec hypertrophie (sclérose ou cirrhose hypertrophique du foie). Arch. de physiol., 1874, i.

4. Hanot, V: Etude sur une forme de cirrhose hypertrophique du foie (cirrhose hypertrophique avec ictere chronique). Paris, 1876.

Parmi les différentes lésions du foie qui ont été englobées sous la dénomination de cirrhose hypertrophique, il en est une qui est vraiment spéciale, et qui se compose des éléments suivants: sclérose extra-lobulaire très-accusée et sans tendance á la rétraction du tissu conjunctif de nouvelle formation; souvent aussi sclérose intro-lobulaire; developpement anormal et catharre chronique des canalicules biliaires.

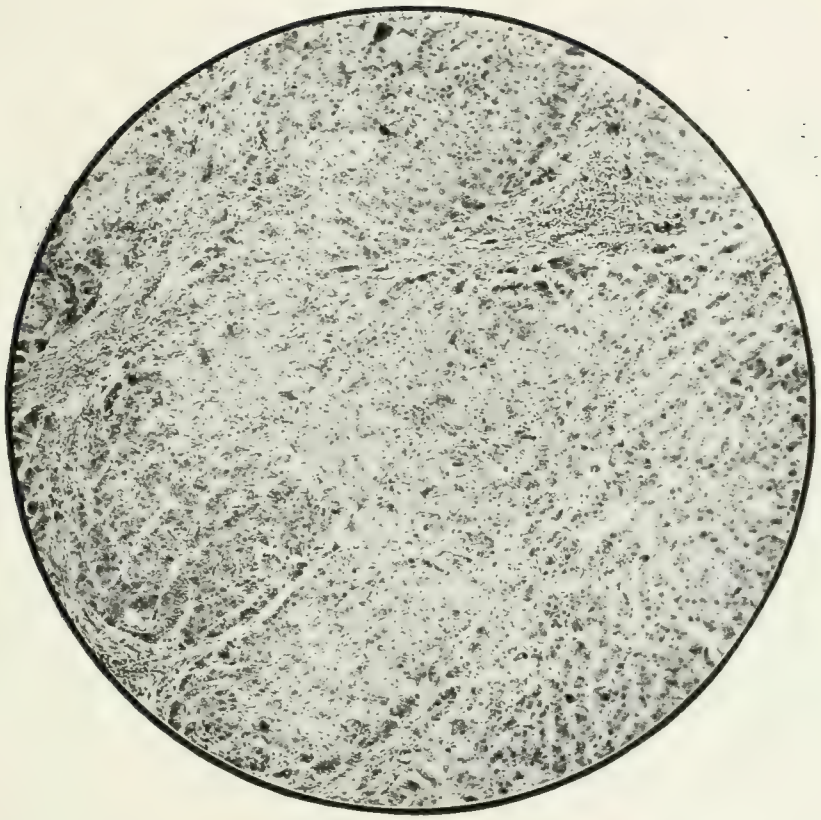


Fig. 3. Perinsular and diffuse sclerosis; exudative and productive hepatitis, from a large but distinctly granular and much jaundiced liver. It shows a variety of lesions: In the center the type is that described by Kretz; at the periphery more a simple periinsular sclerosis. Bile-duct formation and angiocholitis moderate and irregular. Zeiss: Objective 16 mm., ocular 6 mm. The photographs are by Mr. A. J. Martin, artist to the Russell Sage Institute.

that the atrophic cirrhosis took its origin around the radicles of the portal vein, while it was characteristic of the hypertrophic cirrhosis with icterus that it took origin from the bile canaliculæ. In his later monograph on the subject[5] he also emphasizes the good preservation of the liver cells.

In the subsequent discussion of the disease, however, all of these various features which Hanot advanced then, and later in modified form, have been put forward as characteristic by its supporters, notably in order to save the disease from being disregarded as an entity. At present there are a number of different conceptions which are more or less removed from Hanot's ideas, but which still go under the name of Hanot's cirrhosis, all the distinguishing features which were originally and subsequently claimed for this disease having gradually been denied, differently interpreted or found in other forms of cirrhosis.

Hanot, as we have seen, speaks of an interlobular and intralobular cirrhosis, but he himself attached much greater significance to the bile ducts and the origin of the disease from them than to the cirrhotic lesion itself. Subsequent investigators, however, have paid more attention to the distribution of the connective tissue, and have even gone so far as to disregard the involvement of the bile ducts or the origin of the lesion from them, which was particularly emphasized by Hanot. This is especially true of some of the German writers who have accepted the division of cirrhosis into the atrophic and hypertrophic forms. Kaufman,[6] for example, claims as characteristic of Hanot's cirrhosis an extremely fine, diffuse intraacinar connective tissue proliferation, and distinguishes it distinctly from biliary cirrhosis. He further differs from Hanot in stating that the liver cells, that are definitely involved, contain fat and bile pigment, become atrophic and disintegrate with the formation of clumps of bile. He refutes the idea of good preservation of liver cells in hypertrophic cirrhosis as a false interpretation of attempts at regeneration, the latter having been demonstrated through the finding of karyokinetic figures by Prus. Very similar are the ideas, with regard to hypertrophic cirrhosis, which are held by Kretz,[7] who presented two livers before the German Pathological Society in 1905, which he considered typical of Hanot's disease. These cases were characterized by a diffuse intralobular formation of fibrous tissue with considerable atrophy of liver cells, vacuolization, and fatty infiltration, but without capillary

5. Hanot, V.: La cirrhose hypertrophique avec ictère chronique, Paris, 1892.

6. Kaufman, E.: Lehrbuch der speziellen pathologischen Anatomie, 1907.

7. Kretz, R.: Ueber die Abgrenzung der Hanotischen Krankeit gegen die Lebercirrhose mit Ikterus. Verhandlungen der deutschen pathologischen Gesellschaft, 1905.

cholangitis, bile imbibition, or bile thrombi. He, therefore, claims as the distinguishing feature of Hanot's cirrhosis, diffuse intralobular connective tissue growth without capillary cholangitis. It is perfectly clear from the foregoing that, while Kretz speaks of these as Hanot's cirrhosis, this cirrhotic process has not much in common with the ideas which were advanced by Hanot, since the latter paid particular attention to the involvement of the bile ducts and the good preservation of the liver cells. While, then, some of the Germans consider that Hanot's cirrhosis consists of diffuse smooth enlargement of the liver with considerable bile imbibition but no capillary cholangitis, and with a destruction of the liver cells, others follow Charcot and proclaim this a special feature. Rolleston[8] has retained more or less the original typical Hanot description and groups it as biliary hypertrophic cirrhosis.

On the other hand, some pathologic anatomists refuse to recognize any of these features as sufficiently characteristic evidence to permit of the establishment of a definite classification, and with these I must agree. In the first place it has been held that it is characteristic of the so-called hypertrophic cirrhosis to show a lack of contraction due to an even distribution of the cirrhosis, but that this may be found in other varieties of cirrhosis has long been well known. In fact Orth, in his text-book of special pathologic anatomy,[9] describes distinctly cases of smooth atrophic cirrhosis which are due to a very fine uniform distribution of the connective tissue. We have in our collection in this institute a perfect example of such a cirrhosis, which presents a smooth surface as the result of very general and even connective tissue growth which goes hand in hand with a corresponding loss of hepatic substance. On the other hand, there occur cases of cirrhosis with jaundice and marked enlargement of the organ which are distinctly granular as a whole, or in places. How are these to be regarded? The integrity of the liver cells which has been proclaimed as distinctive by some writers can not be held to be in any way characteristic. That feature is open to too much variation, and in every cirrhosis, especially in the typical atrophic forms, one will run across many areas of parenchyma which appear well preserved, to say nothing of the attempts at regeneration which have been shown by Prus. Again, in the forms with marked enlargement and jaundice the parenchyma may be diffusely and severely altered.

With regard to the distribution of the cirrhotic tissue itself, I have already stated that all cirrhoses are intralobular as well as interlobular,

8. Rolleston, H. D.: Disease of the Liver, 1905.

9. Orth, J.: Lehrbuch der speziellen pathologischen Anatomie, 1887, i.

and that a periinsular cirrhosis may at any time become a diffuse intralobular form, if in the course of exacerbations the remaining islands should become injured to such an extent as to allow of an invasion of connective tissue in the form of denser or fine fibrillar bands into them. Finally, the presence of cholangitis and bile duct formation can certainly not be characteristic, for these occur in a number of cases of ordinary cirrhosis, and, as the observations of Kretz show, they may even be absent in cases which otherwise entirely resemble Hanot's cirrhosis. It will, therefore, be seen that the conditions which may be associated with smooth or granular enlargement of the liver and with jaundice are variable and depend upon even more different conditions than were found by the originator of the term. The absence or presence of accessory conditions, like jaundice, ascites and splenic enlargement, can certainly not be utilized in the classification of the cirrhotic lesion, for they depend, as I have outlined above, upon a number of extremely variable pathological lesions which are partly the result of the cirrhotic process itself, and partly of superadded, concomitant, and more or less independent lesions.

At this present stage of our knowledge it seems unjustifiable to insist on the establishment of definite classes of cirrhoses of the liver. We have to deal here with variations which allow such frequent combinations that even clinically we can not gain much by attempting to do this (Fig. 3).

At the symposium on nephritis before the German Pathological Society in 1905 Orth made this remark at the end of the excellent presentations of Ponfick and Fr. Müller on the subject:

Die Lehre von der Nephritis enthält so viele Einzelfragen, dass wir tagelang diskutieren könnten, wenn wir sie alle eigehend behandeln wollten, ich werde mich deshalb nur auf einige kurze Bemerkungen beschänken. Es ist vorher die Hoffnung ausgesprochen worden, dass man heute festere Grundlagen für die Nomenklatur und Systematik der Nierenkrankheiten gewinnen würde, ich glaube nicht der einzige zu sein, welcher das Gefühl einer Enttäuschung empfunden hat, denn wenn man das Resultat von Referat und Korreferat übersieht, so muss man doch sagen, das dasselbe nur ist, dass wir noch fast nichts wissen.

The same sentiments I voice to-day in another subject after a review of our knowledge and some independent study of the inflammations of the liver. Those who believe that morphological investigation of diseases of organs has reached its final results should appreciate that we have really just begun to correlate our morphological experience and develop the variations in its relations. In the future, as in the past, no proper idea of a disease will exist without knowledge of these relations, and both the pathologist and the clinician of to-day should appreciate that proper conception of a disease can stand only on this basis. In the gradual evo-

lution of pathological conceptions a great many previously established sharp lines of demarcation are wiped away, just as they gradually disappeared in zoology after the appreciation of the theory of evolution.

Guided by these considerations, I now use the following very broad classification for diffuse inflammations of the liver, which, although it does not draw rigid lines, allows of the combination of variations which are much closer to the actual anatomical finding.

I have discarded entirely the terms acute, subacute and chronic, which have no place in anatomic terminology, and have substituted descriptive terms which allow proper anatomical conceptions.

1. I speak of degenerative exudative hepatitis, now called acute hepatitis, in cases which are characterized in varying degrees and in varying combinations by cellular exudation taking origin from the portal spaces, by lymphangitis, perilymphangitis and cholangitis, by diffuse parenchymatous and fatty degenerations of the liver cells, and by cytolysis and bile imbibition.

2. I speak of degenerative productive hepatitis in cases which are now grouped as subacute hepatitis, and divide them into two forms: the first with a more rapid loss of substance, grossly appears more or less granular, the second with a much more gradual loss of substance has consequently a more uniform, even distribution of connective tissue and is, therefore, smooth. Both of these may be associated with either enlargement or diminution in size of the liver. They are characterized, microscopically, by a productive cellular inflammation proceeding from the portal spaces, which is periinsular and diffuse: a productive lymphangitis and a productive pericholangitis. It is further characterized in varying degree, and more or less accentuated by, more slowly progressing, irregular, parenchymatous and fatty degenerations and fatty infiltration, cytolysis, bile imbition, and the formation of new, mature and embryonic liver cells (bile ducts).

3. I speak of productive hepatitis, for which I also use the term sclerosis of the liver (which is to be preferred to cirrhosis of the liver), and this is characterized essentially by the formation of mature, periinsular, or diffuse connective tissue formation; depending upon the loss of liver substance and the distribution of connective tissue, it is either granular or smooth and goes along with enlargement or diminution in size of the organ. It further presents very slowly progressing, irregular, parenchymatous and fatty degenerations, and fatty infiltration, cystolysis, little or moderate bile imbibition, and the formation of mature and embryonic liver cells (bile ducts).

30 West Forty-fourth Street.

RECENT ADVANCES IN OUR KNOWLEDGE OF THE UNDERLYING CHEMICAL PRINCIPLES OF DIABETIC ACIDOSIS.

EDWARD H. GOODMAN, M.D.
PHILADELPHIA.

The literature pertaining to acidosis, with especial regard to its bearing on diabetes, has grown in the past few years to such dimensions that it is practically impossible for the general practitioner to keep fully abreast of the recent researches along this line. In reviewing the various articles pertaining to diabetic coma and its underlying condition, acid intoxication, it seems that since 1900 the work has taken on a distinctly new and decidedly more interesting and valuable aspect, mainly due to the energy of German investigators. While most of the work has had to do with the theoretical and experimental side, yet for a thorough understanding of the subject it is absolutely essential that the busy practitioner, not interested generally in chemical research, should be acquainted with the more recent advances in this subject. It has seemed, therefore, fitting to compile reports of recent date, to review the most important papers in detail, to summarize results, and to put these results in a paper of sufficient brevity to acquaint the average physician with the present status of diabetic acidosis.

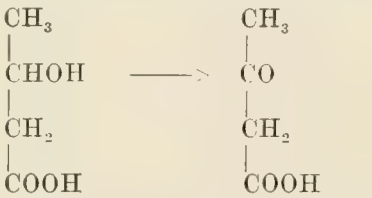
I have chosen, therefore, to limit this paper to a review of reports published from 1900 to the present time, for it is during this period that the most marked advances are seen. For a short history of the subject the reader is referred to Folin's article,[1] in which he concisely summarizes the history of the acid intoxication theory from the time of Boussingault. This part of the question will be omitted, and this paper will be confined solely to the subject of acidosis, with report of the work done on the origin of the acetone bodies, and the probable nature of the effects exerted on the animal organism by their presence. As much technical phraseology will be omitted as is concomitant with a clear representation of the subject, and the endeavor will be made to explain such chemical technicalities as can not well be avoided. I have often observed that, whereas the average practitioner speaks glibly of acidosis, oxybutyric acid, diacetic acid and aceton, he has not the slightest conception of exactly what is meant by these names, but is rather like the

1. The Jour. Am. Med. Assn., 1907, xlix, 128.

Harvard student who, on being asked his definition of "word," replied that it was "a funny sound that means something."

Butyric acid and acetic acid belong to the same group, the type of which is $C_nH_{2n}O_2$. If there is one atom of carbon, there must be two atoms of hydrogen; if two carbon atoms, there must be four hydrogen atoms, and, no matter how many carbon atoms we have in the molecule, the hydrogen atoms are twice as many, while in each molecule there are never more than two oxygen atoms. Acids formed in this way are called fatty, because their higher members occur in the natural fats, and if $C_nH_{2n}O_2$ is remembered a series of fatty acids can be theoretically formed *ad libitum*. Starting, however, with the lowest fatty acid of the series, which must of necessity have but one carbon atom, we find CH_2O_2—H.COOH—or formic acid. The two oxygen atoms, one of the carbon and one of the hydrogen atoms, are always in the combination of COOH, which is called a carboxyl radical. The next acid in series must have the formula, $C_2H_{2x_2}O_2$ in which the N = 2 and we have $C_2H_4O_2$,—CH_3.COOH or acetic acid. The next higher will be $C_3H_6O_2$,—$CH_3.CH_2$.COOH, proprionic acid, and the next above $C_4H_8O_2$—$CH_3.CH_2CH_2$.COOH or butyric acid. Each division of these molecules is named according to its relation in the molecule to the fundamental unchanging carboxyl group. The one nearest to the carboxyl radical is said to be in the alpha position, *a*, and one on the left of the alpha β, and the other γ.

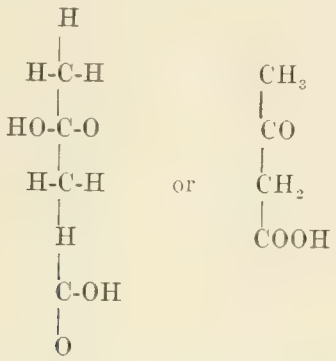
If, by a process of oxidation, there is an oxygen atom inserted in any one of the *a*, β or γ groups, we have formed an oxyacid, and if this oxidation happens to take place in the β position we have formed a β oxyacid, and if butyric acid is so oxidized β oxybutyric acid, $CH_3.CHOH.CH_2.COOH$. Supposing, however, the oxidation does not stop here, but that combustion continues. It has been found by chemists that the oxidation of the fatty acids in the body takes place usually in the β position, furthermore that successive oxidation takes place in the same position; in other words, the substance that is burned up by oxidation in the beta position continues to be oxidized in the same position. Accepting this as a fact, we see the following results:



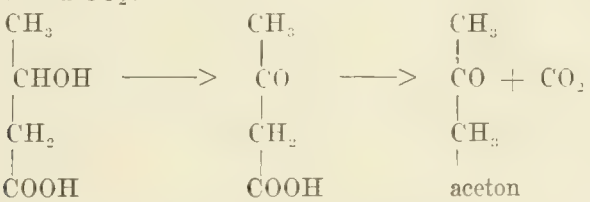
β-oxybutyric acid. Diacetic acid

in which the new body has been formed by deprivation of β-oxybutyric acid of two atoms of H, with the formation of water.

On comparing this newly formed acid with acetic acid, CH_3—COOH, we find it to be made up of two molecules of the latter, minus one molecule of water, thus:



If oxidation continues, and there is still further splitting off, we can imagine the following to take place, the new product arising by liberation of carbon dioxid CO_2:



Aceton is also called dimethylketon, the CH_3 group being called methyl, and if in a molecule the various components are held together with CO, the body is called a keton.

If normally by our qualitative tests we can detect no aceton in a specimen of urine, and we find it in a diabetic case, the organism's power to burn up the aceton is impaired, and it appears as a danger signal referable to some disturbance of metabolism. In such a case, where no diacetic acid is detected, the organism is still sufficiently able to oxidize material past oxybutyric acid, over diacetic acid, but can go no further than aceton. If, however, we find diacetic acid, there is grave disturbance, as the body has now lost the power to oxidize even this, and in such cases oxybutyric acid will generally be found, showing that marked metabolic disorders are present in the organism.

The above is stated merely to review fundamentals concerned in the chemistry of acidosis and is not to be considered as actually taking

place, though such may be the case. With this elementary chemical preface understood, there will be a clearer idea of just what is meant by β-oxybutyric acid, diacetic acid and aceton, and it will probably make more intelligible to the average physician why he dreads the appearance of diacetic acid more than of aceton.

I. ORIGIN OF ACETON BODIES.

A. *From Protein.*—Schwarz[7] thinks that aceton is not derived from proteid, as in a diabetic, during nine days of carbohydrate fasting, there were 27 grams more aceton excreted than in a period of carbohydrate diet, and the nitrogen output was no higher in the first period than in the second, which would be the case if the protein tissue were being broken down. He thinks that aceton is an expression of increased fat destruction, as it is seen when the organism has to provide heat for itself, as in carbohydrate abstinence and in hunger. Blumenthal and Neuberg[3] state that Guckelberger and Cohn had obtained aceton from casein, and to study the question of origin of aceton from proteid they investigated the action of iron salts on gelatin, and, finding that aceton was produced, they proposed the hypothesis that the iron in the body has some effect on oxidation and probably plays a rôle in the formation of aceton. Schwarz,[4] shortly after this, criticized the work of Blumenthal and Neuberg, his main objection being that they should have demonstrated β-oxybutyric acid, and if aceton is found it must be obtained by oxidation of the former. He also states that Blumenthal and Neuberg have not shown anything new, as Cotton, in 1899, had obtained aceton from gelatin, fibrin and casein by distilling with hydrogen peroxid.

Orgler[5] produced aceton from crystallized egg albumin. Neuberg and Blumenthal[6] think that aceton may be formed from all three foods —protein, carbohydrates and fats—that the last does not account for all the aceton, and they believe that it is formed from protein tissue. Their later work is merely a confirmation of their former paper.[3] Baer[7] seems to hold the view that aceton is not formed from albumin, for so long as the animal remains in nitrogen equilibrium there is no increase of aceton, but, when this is disturbed, symptoms of acidosis appear. Satta,[8]

2. Centralbl. f. Stoffwechsel-u. Verdauungskr., 1900, p. 2.
3. Deutsch. med. Wchnschr., 1901, p. 6.
4. Id., 251.
5. Hofmeister's Beitr., 1901-2, i, 583.
6. Id., 1902, ii, p. 238.
7. Arch. f. exper. Path. u. Pharmakol., 1903-4, li, 271.
8. Hofmeister's Beitr., 1904-5, vi, pp. 1 and 388.

in studying the condition of aceton formation, does not believe much in its origin from protein for the following reasons:

(*a*) There is no relation between nitrogen and aceton output.

(*b*) In diabetics, nitrogen equilibrium, even a storing up of nitrogen, can be obtained without any decrease in the excretion of aceton bodies being seen.

(*c*) Mangus Levy found that in one case 262 grams of protein was used up in three days, and 342 grams of β-oxybutyric acid were excreted, which is more than could be accounted for by supposing its origin to have been from albumin.

(*d*) The objection that a proportion between nitrogen decomposition and aceton excretion can not be made because the breaking down of the protein molecule does not go to the end products, is contradicted by the relation between sulphur and phosphorus. It has been found that there is a constant ratio between these two, but none between aceton and sulphur.

(*e*) Rosenfeld, Hirshfeld and others have reported that much albumin ingestion cuts down acetonuria.

(*f*) It is very difficult to understand why small amounts of carbohydrates, which only inhibit the albumin decomposition to a slight extent, should cause pronounced disappearance of aceton.

Satta regards it as proved that the greater amount of aceton does not come from albumin. He remarks that the mere fact of having obtained aceton outside the body by the use of strong oxidizing agents has not much value, because the amount of aceton is small, no other substance beside aceton is obtained, and because a similar procedure in the organism is not possible. Satta suggests that the aceton-forming bodies be classified as ketoplastic and antiketoplastic. The first contains those bodies which are regarded as the immediate sources of fatty acid aceton, and the second are inhibiting stuffs which prevent the formation of aceton or from which no aceton is formed (carbohydrates).

Borchardt[9] investigated the action of monamido acids on the aceton output, and, knowing that protamins and histons contain but traces of monamido acids, egg albumin a moderate amount, albumin of pancreas much, and casein a great deal, it would be expected that protamins and histons would have no antiketoplastic action, the action of the others being dependent on the amount of the monamido acids present in the molecule. At present, aceton formation is supposed to be from fat which contains ketoplastic groups—fatty acids—and antiketoplastic groups—glycerin. As the antiketoplastic group can be removed

9. Arch. f. exper. Path. u. Pharmakol., 1905, liii, 388.

from the protein molecule, the question naturally arises, Has the protein molecule, groups from which aceton bodies can be formed? If so, then those proteins which contain no antiketoplastic groups, as protamin and histons, should give an increased amount of aceton. For those proteins which contain only a moderate amount of inhibiting stuffs the result is not known, as the unknown ketoplastic and not wholly known antiketoplastic groups (leucin and monamido acids) are antagonistic, and it is not known which is the more powerful. But it is to be expected here that egg albumin poor in monamido acids has rather a ketoplastic action than the leucin-rich casein, whereas the proteid of the pancreas, corresponding to its leucin percentage, stands between both.

Borchardt states that Magnus Levy[11] found such quantities of oxybutyric acid in the urine of cases of severe diabetes, that its origin from protein or fat bodies did not account for it, and another source of aceton must be sought. The breakdown of high fatty acids to bodies with two or three carbon atoms (Magnus Levy) to form aceton is from a chemical standpoint improbable, but can be neither proved nor controverted. Borchardt used carp's eggs which contain 81 per cent. of nucleic protamin, calf's thymus containing 70 per cent. of nucleic histon, native and coagulated egg albumin, pancreas and nutrose, and found that feeding on protamin, histon and egg albumin caused a marked increase of aceton output, after pancreas he was uncertain, and with casein the aceton was diminished. His results do not correspond with Lüthje's[12] experiment on diabetics.

Lüthje found after nutrose that the nitrogen, sugar and aceton were increased, but with thymus all sank, giving as an explanation that nutrose is burned up rapidly in the organism and consequently there is a high nitrogen output, its carbohydrate-forming groups are very great, and in diabetes this causes more sugar, and, as the unused carbohydrate formers do not act antiketoplastically, the aceton is increased. Calf's thymus is more slowly used up, hence a diminution of nitrogen, and, as it contains no carbohydrate-forming groups, there is no sugar produced, and this would account for increase of aceton. The sinking of aceton means that casein contains antiketoplastic substances (carbohydrate formers), which, being stored up, are broken down in the after period and act during the period of thymus feeding as inhibiting stuffs. v. Noorden found that the different albumin bodies acted differently on the excretion of aceton, the vegetable protein having less ketoplastic

11. Quoted by Borchardt.
12. Ztschr. f. klin. Med., xxxix.

power than egg albumin. Borchardt found that when nutrose was substituted by three eggs the aceton increased, and this effect was more pronounced with 200 grams of meat.

Owing to our lack of knowledge of the split products of albuminous breakdown, the meaning of these experiments is obscure. The fact that the protein bodies which are rich in carbohydrate producers (casein, pancreas) have no ketoplastic action, is in favor of the view that the carbohydrate-forming groups of the protein molecule are inhibiting stuffs; together with these antiketoplastic groups there is a ketoplastic group. Better known than the mother substances of aceton are the antiketoplastic substances—carbohydrates, glycerin, lactic acid, and, according to Borchardt's experiments, probably a group in the protein molecule.

As stated above, Magnus Levy found that no one group (neither fat nor proteid) could be regarded as being the mother substance of aceton. Of course, the fatty acids are now known positively to be a source of aceton, but it is not known what other substances have the same property, but Borchardt believes that there is a ketoplastic group in albumin. The percentage of monamido acids in protamin, histon, egg albumin, pancreas and casein is lowest in the order of substances named, as are also the carbohydrate-forming groups, whereas the ketoplastic power increases from casein up to protamin. Protamin, histon and egg albumin increase the aceton output, the pancreas has no influence, and casein acts antiketoplastically.

Borchardt gives another explanation, however. During pepsin and trypsin digestion, protein is broken down to so-called antigroups, very resistant in nature, and to hemigroups, very readily broken up further. The first are forerunners of polypeptids and are ketoplastic; the latter are antiketoplastic. Casein is rich in hemigroups and consequently is easily broken down; whereas protamin is hardly digested by trypsin at all, and only exceptionally is broken down to proton. This theory means more than that the antigroup contains certain monamido acids, as leucin, for other amido acids, as glykopoll, belong exclusively to the antigroup. The different action of the same monamido acid in the hemi- and antigroups can be explained by supposing different union in the proteid molecule.

Which of the theories is correct has not been decided, but it is certain that the protein molecule contains ketoplastic and antiketoplastic groups, and that the proportion of one to the other determines its influence in aceton excretion.

In favor of albumin as a source of aceton, Baumgarten and Popper[13] report that aceton was a constant accompaniment of extrauterine pregnancy with hemorrhage into the peritoneal cavity. Based on this clinical observation they believe that aceton is derived from the proteid tissue in blood.

In discussing the ketoplastic and antiketoplastic bodies, Halpern and Landau[14] suggest that the antiketoplastic bodies prevent the formation of aceton by setting oxygen free during their breaking down, and that by this means aceton is oxidized. For instance, if the body, for any reason, can not use up carbohydrates, there is an increased output of aceton bodies through the lungs and kidneys, the amount depending on the degree of disturbance. Borchardt is quoted by them as saying that the antiketoplastic action depends on the amount of amido acids contained in the protein molecule, for leucin and alanin do not cause increase of aceton. Therefore, the more amido bodies the protein molecule contains, the greater its antiketoplastic property. This view of Halpern and Landau does not seem to the writer tenable, as carbohydrates, which contain no amido groups, are the most powerful of the antiketoplastic bodies.

Later, in 1907, Borchardt and Lange[15] set about to answer three questions: (1) Which components of fat and albumin act as augmentors and which act as inhibitors of aceton? (2) Are the substances which increase aceton output those from which oxybutyric acid or aceton can be derived directly? (3) Which substances are the direct source of aceton? I shall here refer only to these problems in their bearing on the question of aceton formation from protein.

Does the protein molecule contain augmenting or inhibiting aceton factors? Borchardt affirms this, but without going into the nature of these substances. At that time it was thought that monamido acids inhibit aceton output, because protein poor in monamido acids increase aceton, and *vice versa*. The question is now, What chemical substances (referring only to fatty acids and their derivatives) can be transformed into oxybutyric acid or directly into aceton, and, finally, which of these substances occur in food, fatty or protein; in other words, which may be considered as sources of aceton bodies?

Borchardt and Lange decided to repeat the experiment of Embden and others with perfusion of the liver, by feeding the same substances to men. As these experiments of Embden et al. bear mostly on the forma-

13. Wien. klin. Wchnschr., 1906, p. 334.
14. Ztschr. f. exper. Path. u. Therap., 1906, iii, 466.
15. Hofmeister's Beitr., 1907, ix, 116.

tion of aceton from fat, I shall mention this part of their paper under that division of my subject dealing with this side of the question. The experiments of Borchardt and Lange will also receive the consideration which their excellent work deserves, but their conclusions may be briefly stated as follows: That those amido products of albumin digestion which lead to formation of aceton do so by being converted into their corresponding fatty acids and are oxidized to aceton in this way.

There is, of course, ample proof that aceton can be formed from protein tissue, but, as this formation always takes place over fatty acids, protein may, in the light of investigations previously quoted, be considered not as a source of aceton *sui generis,* but merely as a source of amido acids which, in their turn, by breaking off of the amido group are converted into fatty acids and then into aceton.

B. *Origin of Aceton from Carbohydrates.*—The only reference I have found to this side of the question is a work by Neuberg and Blumenthal.[6] They quote Emil Fischer as believing that carbohydrates are derived from fat, and Magnus Levy, who believes that in the breaking up of hexoses, lactic acid is first formed according to the following equation: $C_6H_{12}O_6 = 2CH_3—CHOH—COOH$. This acid goes easily into formic acid and acetaldehyd, $CH_3—CHOH—COOH = H.COOH + CH_3.CHO$. With this as a working basis, Neuberg and Blumenthal develop a very pretty hypothesis. By condensation of two molecules of acetaldehyde an aldol is formed, $2CH_3.CHO = CH_3—CHOH—CH_2—COH$ (β-oxybutyraldehyde), and this, in turn, by shoving of oxygen in between molecules or by splitting off of water, forming crotonaldehyde—$CH_3.CHCH.COH$—and then adding it, butyric acid is formed. By repeated condensation of aldol and crotonaldehyde and subsequent reduction, the higher fatty acids may be supposed to be formed. They think that the question of formation of aceton from fat may be equally well regarded as a question of aceton formation from carbohydrates, also that the hypothesis is rather stretched in supposing that fatty acids must be broken up to butyric acid and this, in turn, be subjected to a succession of complicated reactions before aceton can be formed; so they prefer to believe that the same substances form aceton as form fat, namely, carbohydrates.

The above view has not been generally accepted; in fact, I know of no work published which deals with this novel theory of Neuberg and Blumenthal. *In vitro* and *in vivo* are two entirely different questions, and until laboratory experiments can be reproduced, in a measure, in the body, their importance in metabolism problems is somewhat vitiated. There is no proof that sugar or any carbohydrate increases aceton, and

thousands of instances where acetonuria has been held in abeyance by the ingestion of sugar. My personal belief is that the question of the derivation of aceton from carbohydrate has but little importance, if any.

C. *Origin of Aceton from Fat.*—Here it is that we meet with the probable solution of the whole question of the source of the aceton bodies. It has been known, or at least conjectured, for a great while that fat or some component of fat has the most to do with the formation of aceton, and, to my mind, the work of Embden, Salomon, Borchardt and others has put the subject on a very sound basis from which there does not seem to be any probability of its being dislodged.

Geelmuyden (quoted by Hagenbeck[16]) found that fat containing no low fatty acids (beef tallow) diminished and prevented aceton, whereas butyric acid increased it, and stated that the varying acetonuria on fat diet, for instance butter, depended on the amount of the volatile fatty acids present. Hagenbeck took up this particular point, using the calcium salt of butyric acid. His object was to study aceton, so he disregarded β-oxybutyric acid entirely. He confirmed Geelmuyden's observation, and thinks the presence of aceton is dependent on the lower fatty acids. He states, furthermore, that *in vitro* butter, plus potassium permanganate and sodium hydroxid, gives rise to aceton.

Schwarz[17] found that in health there was no increase of aceton after fat ingestion, but, in diabetes, fatty diet, especially butter, caused marked increase, and this increase was higher if aceton was high before the experiments.

Waldvogel and Hagenberg[18] studied the effect of over-nourishment with fat and fatty acids on healthy organism, selecting themselves for study, and found that, despite abundant food, with certain amounts of fat and fatty acids, the aceton excreted was normal. Just the opposite view is held by Schumann-Leclercq,[19] who states that fat must be regarded as the principal cause of aceton, no matter if there is disturbance of body or food fat, or whether the patient is fasting, on fatty diet, or on meat diet, and, furthermore, that carbohydrates inhibit aceton by protecting the labile fat from combustion to aceton.

Mohr and Loeb[20] found after 200 grams of butter that β-oxybutyric acid was increased, but there was no increase after 300 grams sesame oil, which they attribute to absence of volatile fatty acids. By increasing

16. Centralbl. f. Stoffwechsel-u. Verdauungskr., 1900, p. 33.
17. Id., p. 2.
18. Ztschr. f. klin. Med., 1901, xlii, 443.
19. Wien. klin. Wchnschr., 1901, p. 237.
20. Centralbl. f. Stoffwechsel-u. Verdauungskr., 1902, p. 193.

or diminishing fatty food they observed an increase or diminution in oxybutyric acid, whereas the aceton was unaffected. They also noticed that both aceton and oxybutyric acid were diminished after glycerin, and confirmed Rumpf's observation of increase of β-oxybutyric acid after sodium butyrate, only they used pure butyric acid neutralized with NaOH.

Neuberg and Blumenthal[6] refute the idea of aceton formation from fat and contend that if it takes place it is due to bacterial activity in the intestine. Johannes Müller was the champion of this view, but Lüthje, quoted by Neuberg and Blumenthal, discovered in a diabetic girl that calomel had no effect on aceton and diacetic acid. The strongest proof that Neuberg and Blumenthal offer is that it is only per os that butyric acid increases the output of aceton, the subcutaneous injections having no effect.

It is supposed that the following reactions take place in the bowels,

CH_3-CH_2-CH_2-COOH — Butyric acid.

CH_3-$CHOH$-CH_2COOH ——→ β-oxybutyric acid.

CH_3-CO-CH_2-COOH

CH_3-CO-CH_3

but Neuberg and Blumenthal state that the pure chemical oxidation of butyric acid with nitric acid takes place in the gamma position, and succinic acid, COOH—CH_2—CH_2—COOH, is formed, or, if oxidation with chromic acid, there is a shattering of the molecule with the formation of CO_2 and acetic acid. They prefer to believe in the carbohydrate origin of aceton, as previously stated.

Baer[7] has found that when much albumin is ingested the acidosis is diminished, much carbohydrate causes it to disappear, while much fat, especially the lower fatty acids, increases it. On a mixed diet, however, he states that fat ingestion causes no acidosis; the few exceptions that Waldvogel found are to him not proof against this view, but Baer believes that the degree of acidosis, that is the amount of aceton bodies excreted, is due not so much to the food ingested as to the disease itself, as the fatty acids or esters are in sufficient quantity in the body to account for all the aceton.

Schwarz,[21] in a study of diabetes, found a great difference in aceton output in (a) healthy people and non-diabetics and in (b) diabetics. In the former, he found only a very slight increase of aceton after fat, and this was seen only when the fat intake was very large, but if the carbohydrates are withdrawn and the healthy subject is starved, making of him an acetonuric, butter is found to increase the aceton. These subjects can not, however, be classed as healthy when subjected to such

21. Deutsch. Arch f. klin. Med., 1903, lxxvi, 233.

abnormal conditions. As aceton has been found in pregnancy (Stolz[22]), typhoid fever (Bernert[23]), phosphorus poisoning (Walko[24]), also after chloroform and other narcotics, in inanition, and carcinoma of the gastrointestinal tract, it seems to be an expression of abnormal fat destruction.

In diabetics Schwarz has found aceton increased with all sorts of fats, although pig's fat did not exert so marked an influence as did other fats. The higher fatty acids were found to increase the aceton, though this increase was not as noticeable as after the lower fatty acids.

An increase of aceton after ingestion of acids of the oleic acid group, $C_nH_{2n}—2O_2$, is rarely seen, and Schwarz supposes, as these acids are much more sensitive to oxidative processes outside the body than are the fatty acids, that they are more easily burned up in the body. Aceton can be formed from body fats over the butyric acid route, either by successive splitting off of CO_2 or by breaking up of long C chain, and the four carbon chain is oxidized in the β position, which is a peculiarity of the organism, as by chemical means this can not be brought about. Schwarz made a few experiments with β-oxybutyric acid in health and in diabetes, and found that a dog could use three grams pro Kg. body weight without a trace appearing in the urine. In mild diabetes, an amount which a healthy person could use up without an effort produced an increase of aceton only, whereas in severe diabetics not only aceton was seen, but also unchanged β-oxybutyric acid. Aceton was not completely used up by normal man.

As a result of his studies Schwarz makes the following conclusions:

1. In health fat causes only slight increase of aceton.

2. It is probable that in many conditions, such as starvation, phosphorus poisoning, narcosis and coma, aceton is due to melting of body fat, although not all wasting diseases are accompanied by aceton.

3. In severe diabetes, aceton may be derived from the fat of food or from body fat if the carbohydrate intake is cut down. The intestinal canal does not seem to play an important rôle in aceton formation.

4. The total aceton output is subject to variation even with constant diet, and the amount of β-oxybutyric acid excreted is very large; it can be as high as 70 per cent. or even more.

5. The higher fatty acids do not increase the aceton bodies as do the lower acids; the acids of the oleic acid group least of all.

22. Arch. f. Gynäk., 1902, lxv, 531.
23. Ztschr. f. Heilk., 1902, xxiii, 113.
24. Quoted by Stolz (loc. cit.).

6. Butter increases the aceton more than do the animal fats. The action of oils is dependent on the presence of fatty acids.

7. In normal people, but especially in severe diabetics, ingested β-oxybutyric acid is insufficiently combusted if no carbohydrates are taken, whereas aceton is badly used up in any case, and can not be regarded as an intermediary metabolic product, although β-oxybutyric acid may be.

8. Lipemia is higher in severe diabetes than in mild cases, is seen even with absence of fat from the diet, apart from coma, and can be associated with no symptoms for a long time, but is always associated with excretion of aceton bodies.

9. Alimentary lipemia is seen in severe cases of diabetes after large amounts of fat.

Geelmuyden[25] thinks that the aceton bodies are formed in large amounts in health, but are changed again to other substances. In health, diacetic acid and oxybutyric acid are well burned up by organisms, but aceton is not; hence it should not be regarded as a product of intermediary metabolism, as are the other two acids. These he regards as normal intermediary products, but, being burned up as soon as formed, are not excreted. The fact that carbohydrate inanition is the sole cause of acetonuria is explained by Geelmuyden on the belief that carbohydrates unite with aceton in intermediary metabolism by synthesis as glycuronic acid, which process is necessary for the further change of the aceton bodies; failure of this synthesis or limitation of the same causes a collection of aceton bodies and their subsequent excretion. He regards fat as the principal mother substance of aceton.

Knoop,[26] realizing the difficulty of studying the intermediary cleavage products of fatty acids, states that the only way to study this properly, is by combining these acids with a substance which is attacked with difficulty by the organism, as otherwise the fatty acids are broken down so completely and so quickly that no intermediary products can be found. He recalls the fact that tyrosin, phenylalanin and *a* aminocinnamic acid are completely oxidized in the body; phenylproprionic acid and cinnamic acid are oxidized to benzoic acid, whereas phenylacetic acid and its derivatives, mandelic acid and phenylamido acetic acid, are unchanged, with the exception of the last, which simply has its NH_2 group replaced by the hydroxyl radical OH. It will be recalled by consulting Table I that all these substances have the benzene (C_6H_5) group in common; so why should the other bodies be oxidized and not the phenyl-

25. Ztschr. f. physiol. Chem., 1904, iv, 128.

26. Hofmeister's Beitr., 1904-5, vi, 150.

TABLE 1.

Ingested.	Acid.	Excreted.		Change Observed.
Benzoic acid	$C_6H_5.COOH$	Benzoic acid	$C_6H_5.COOH$	Unchanged.
Phenylacetic acid	$C_6H_5CH_2.COOH$	Phenylacetic acid	$C_6H_5.CH_2.COOH$	Unchanged.
Mandelic acid	$C_6H_5CHOH.COOH$	Mandelic acid	$C_6H_5.CHOH.COOH$	Unchanged.
Phenylamidoacetic acid	$C_6H_5.CH(NH_2).COOH$	Mandelic acid	$C_6H_5.CHOH.COOH$	Detritrified.
Phenylproprionic acid	$C_6H_5.CH_2CH_2.COOH$	Benzoic acid	$C_6H_5.COOH$	
Phenyl β-lactic acid	$C_6H_5.CHOH.CH_2.COOH$	Benzoic acid	$C_6H_5.COOH$	Oxidized at β-carbon atom.
Phenyl β-ketoproprionic acid	$C_6H_5.CO.CH_2.COOH$	Benzoic acid	$C_6H_5.COOH$	
Cinnamic acid	$C_6H_5.CH:CH.COOH$	Benzoic acid	$C_6H_5.COOH$	
Phenylalanin	$C_6H_5.CH_2.CH(NH_2).COOH$	Disappears		
Phenyl α-lactic acid	$C_6H_5.CH_2.CHOH.COOH$	Disappears		Completely oxidized.
Phenyl α-ketoproprionic acid	$C_6H_5.CH_2.CO.COOH$	Disappears		
α-Amido cinnamic acid	$C_6H_5.CH:C(NH_2).COOH$	Disappears		
Phenylbutyric acid	$C_6H_5.CH_2.CH_2.CH_2.COOH$	Phenylacetic acid	$C_6H_5.CH_2.COOH$	Oxidized at β-carbon atom.
Benzoyl proprionic acid	$C_6H_5.CO.CH_2CH_2COOH$	Phenylacetic acid	$C_6H_5.CH_2.COOH$	
Phenyl iso crotonic acid	$C_6H_5.CH:CH.CH_2.COOH$	Phenylacetic acid	$C_6H_5.CH_2.COOH$	Oxidized at δ-carbon atom.
Phenylvaleric acid	$C_6H_5.CH_2CH_2CH_2CH_2.COOH$	Benzoic acid	$C_6H_5.COOH$	

acetic acid or its derivatives? It is not possible that phenylproprionic acid is oxidized to phenylacetic acid, or else this substance would appear in the urine, or at least one of its derivatives, so Knoop believes that the oxidation process takes place not in the α position, but at the β carbon atom. Whether the oxidation of the side chain is the rule or not, Knoop proposes to decide by studying the higher homologs of phenylproprionic acid or of its derivatives.

Without going into detail with Knoop's experiments with individual acids, it will suffice to reproduce his table with a short summary of his conclusions as given by Folin.[1]

"When ordinary fatty acid derivatives are linked to the benzene group, C_6H_5, and the resulting compounds are fed to dogs, the fatty acid chains are broken down by oxidation of the carbon atom third from the end of the chain and the intervening two carbon atoms are split off. In other words, not one, but two carbon atoms are split off at a time, and only so many are split off as can be taken two at a time and still leave, at least, one carbon atom attached to the benzene ring.

"If the fatty acid side chain attached to the benzene radical contains only one or only two carbon atoms, these can not be split off, and such compounds will pass through the organism unchanged or but little altered. With three or with four carbon atoms in the chain two can be split off, while with five or six carbon atoms in the chain four can be removed."

Satta[8] thinks that there is no doubt that fatty acids are the source of aceton, but questions if the splitting of fatty acids takes place normally over β-oxybutyric acid or aceton. In favor of the view that normally this does not occur, he writes as follows:

1. The constant excretion of small quantities of aceton by the kidneys and lungs leads one to believe that aceton is a normal product of metabolism, although this does not mean that the aceton thus formed is formed in the same way as the aceton seen under pathologic conditions. The small amount of aceton and absence of β-oxybutyric acid in normal urine makes one think that either the quantity of the precursors of aceton is small, or else the aceton is not derived from the same source as pathologic aceton; that is, is not formed from oxybutyric acid. The latter is never seen normally, even when the excretion of aceton exceeds the usual amount, despite carbohydrate ingestion, and L. Schwarz found no oxybutyric acid in normal persons, even when aceton was as high as 0.61 grams a day. Satta says that β-oxybutyric acid is not a normal metabolic product and that the normal aceton comes from another mother substance and not from β-oxybutyric acid.

2. It is impossible to cause the disappearance of aceton. Even with much carbohydrate ingestion the normal aceton can not be made to disappear, while under the same conditions the aceton output is markedly diminished.

3. The normal organisms can burn up quite readily β-oxybutyric acid and diacetic acid, although there have not been many experiments with the latter, while, on the other hand, the oxidation of aceton is not only deficient in diabetics, but also in normal individuals.

While to Satta's mind the fact that abnormal aceton is a product of fat splitting, yet this does not help in explaining the presence of normal aceton. If a person is fed on excessive protein and carbohydrate food the normal aceton still appears. In this case no fat has been ingested and no fat is being broken down. On the other hand, by withdrawing carbohydrates from the food, forcing the organism to draw on its own heat-supply, aceton is increased, and all that is necessary in order to diminish it again is the ingestion of carbohydrates.

In this connection it may be well to consider how these antiketoplastic bodies act. One view is that these stuffs act directly or indirectly, increasing the oxidation of β-oxybutyric acid to aceton, but this is scarcely possible, as β-oxybutyric acid, when ingested, is burned up with no increase of aceton, and, as aceton is poorly attacked, the oxidation of β-oxybutyric acid over diacetic acid and aceton can not take place. Another view advanced by Geelmuyden[25] is that a synthesis takes place between β-oxybutyric acid and carbohydrates, probably glycuronic acid, by means of which the aceton formation is hindered, and failure of this synthesis on carbohydrate diet is expressed by acetonuria. Satta believes that this synthesis does not take place between β-oxybutyric acid and carbohydrates alone, but also between the precursors of β-oxybutyric acid and carbohydrates. That the pairing of the former is not with glycuronic acid alone is shown by the fact that levulose and pentose act antiketoplastically, but do not form glycuronic acid. Still a third view is that, in the presence of these antiketoplastic bodies, no β-oxybutyric acid is formed from fat, consequently no diacetic acid and aceton.

Against the intestinal origin of aceton formation Satta makes the following objections:

1. Purges do not diminish aceton, but often have the opposite effect, and even salol and benzol, two well-known intestinal antiseptics, are frequently followed by increase of aceton.

2. The gastrointestinal tract does not contain considerably more aceton than do other organs, and Magnus Levy found less aceton in the gastrointestinal tract of a diabetic than in the other organs.

3. If aceton is formed in the gastrointestinal tract, there should be a direct relation between proteid decomposition and aceton, but Satta found that sulphur diminished when aceton was increased and went up if aceton remained constant or sank.

4. The aceton of inanition could not be explained on this basis, for, if nothing is taken into the stomach, how can aceton be formed from protein decomposition in the intestines? Müller, however, states that albumin is still decomposed, but against this it may be stated that:

(a) Inanition acetonuria increases while the amount of gastrointestinal contents diminishes.

(b) If the stomach and intestines are analyzed, it is found that only 16.23 grams of β-oxybutyric acid can be obtained.

(c) If it is believed, as Müller himself holds, that aceton is derived from fat, then this militates against the gastrointestinal theory, as fat is scarcely ever present.

(d) The immense amounts of aceton seen in coma, and when patient has fasted three and four days, lead one to think that the building place of aceton is within the cells, although in which organ is still undecided. Satta suggests that in those organs having the most to do with the utilization of fat, namely, the glandular organs, there should be found more oxybutyric acid and its descendants (Knoop's experiments show that a fatty acid molecule through successive oxidation in the β position, finally yields one molecule β-oxybutyric acid).

Baer[27] studied the effect of glycuronic acid on acidosis, working on the idea of Geelmuyden,[25] that glycuronic acid is the underlying feature of acidosis. He gave dogs camphor in order to cause increase of paired glycuronic acid, and hence withdrawal of this acid from metabolism, but he found no increase of aceton or of β-oxybutyric acid. In fact, he produced a diminution of β-oxybutyric acid and aceton, but in order that the objections should not be raised, that, as camphor is an excitant, it might cause increased combustion of aceton bodies, he substituted chloral hydrate. In this case he found the acidosis to be diminished and that oxybutyric acid almost disappeared. As the dog was somnolent, he again made a substitution with morphin, and, finding that there was an increase of acidosis, he gave chloral hydrate again, with an immediate diminution of aceton.

If by withdrawal of glycuronic acid the acidosis is diminished, or if in excretion of glycuronic acid, with glycosuria, no acidosis appears, it would seem that glycuronic acid is itself the source of aceton bodies. Indeed, a similar idea was advanced by Flückiger as follows:

27. Ztschr. f. klin. Med., 1905, lvi, 198.

$COH(CHOH)_4.COOH$ (glycuronic acid) $= C_4H_8O_3$ (oxybutyric acid) $+ CH_2O_2$ (formic acid) and CO_2, but this theory does not assist us much in the solution of the question, as we know that aceton may be derived from many sources.

Baer is inclined to believe that in fasting glycuronic acid is not derived from pre-existing carbohydrates, but from protein, as suggested originally by Loewi or from other groups having nothing to do with carbohydrate production, perhaps from mother substance of aceton bodies. It is not improbable that decomposition products arise together with glycuronic acid and by further combustion cause diminution of acidosis.

Borchardt,[28] in discussing the sources of aceton bodies, says that fat is ketoplastic and glycerin antiketoplastic. As fat is resorbed from intestines and appears in chyme as neutral fat by synthesis with glycerin derived from the body, he suggests that the ketoplastic action of fat is due not so much to the acid formers as to withdrawal of the antiketoplastic substance, glycerin.

This view is further supported by the fact that fatty acids injected subcutaneously are not ketoplastic. Geelmuyden found no acetonuria after two grams of butyric acid were given in that way. Waldvogel found no aceton bodies after the administration of olive oil, and it is now settled that fat without fatty acids diminishes aceton body excretion.

The difference in action of ingested fatty acids and injected fatty acids is very easily explained by Borchardt as follows: If given per os, there is a synthesis with the glycerin of the body, with the formation of neutral fat-tri glycerids of the ingested fatty acids. This glycerin, being derived from the body, has no power to form sugar (which ordinarily it accomplishes very easily), and it loses its antiketoplastic property on this account.

It will be remembered that carbohydrates lessen the output of the aceton bodies. If the fat is injected subcutaneously, there is no synthesis with glycerin, no withdrawal of glycerin from the body, the glycerin retains its sugar-forming antiketoplastic property, and there is no increase of aceton. It is this withdrawal of glycerin from the body and not the action of the fatty acids themselves that accounts for the ketoplastic action ascribed to the latter. Although apparently in favor of this ingenious view, Borchardt remains non-committal, preferring that time and future experiments should furnish the proof. This work, to my mind, refutes the arguments of Neuberg and Blumenthal.[6]

Embden and Kalberlah[29] refer to a previous series of experiments in

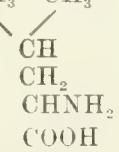
28. Centralbl. f. d. ges. Physiol, u. Path. d. Stoffwechs-u, 1906, viii, 121.
29. Hofmeister's Beitr., 1906, viii, 121.

which they showed that the liver was the chief building station of aceton. Embden, Salomon and Schmidt[30] go still further and study from what substance aceton is formed. As their work is of great interest, I have chosen to review it at length. The above authors start their work with two postulates:

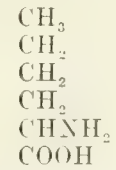
1. If aceton is a breaking down product of a substance, it ought to display the same characteristic structure as the mother substance from which it is derived.

2. Appearance of aceton after perfusion leads to the idea that addition of substances to the blood ought to increase aceton, as it is bound up with difficulty in the liver.

They added glycocoll, alanin, glutaric acid and asparagin to perfused blood, but found no increase in aceton. After leucin, however, there was a marked increase and they suggest that aceton may be derived from the iso propyl group. If that is so, then an isomeric compound of leucin should

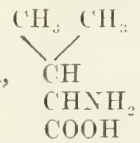


not give it, and they used *a* amido normal caproic acid,



and found, as they supposed, that the aceton remained within the limits of that found after perfusion of liver with blood, so that the increase of aceton is probably not due to the iso propyl group.

They tried the next lower *a* amido iso valeric acid,



which did not cause increase of aceton, for there was neither a splitting of carbon from carboxyl group, nor a splitting between the *a* and *β* carbon atom.

v. Noorden and Embden suggested that there is a splitting between the *β* and *γ* carbon atom, which would explain the aceton seen after leucin, and not after amido valeric acid, as such splitting would not produce aceton here.

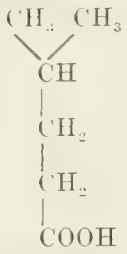
Phenyl proprionic acid, $C_6H_5.CH_2CH_2COOH$, and cinnamic acid, $C_6H_5.CH:CH.COOH$, are changed in the organism to benzoic acid,

30. Id., p. 129.

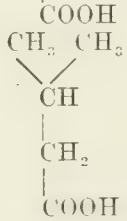
$C_6H_5.COOH$, and hippuric acid, $C_6H_5CO:NH.CH_2COOH$, and it will be remembered that Knoop found that γ phenyl butyric acid was split up to phenyl acetic acid, $C_6H_5CH_2COOH$, and appears as such, whereas phenyl valeric acid, $C_6H_5(CH_2)_4.COOH$, goes into hippuric acid. Also it was found that β-phenyl lactic acid, $C_6H_5.CH_2.CHOH.COOH$, and benzoyl acetic acid, $C_6H_5CO.CH_2COOH$, appear as hippuric acid; benzoylproprionic acid, $C_6H_5.CO_2CH_2CH_2COOH$, and phenyl iso crotonic acid, and $C_6H_5CH:CH.CH_2.COOH$, as phenylacetic acid, and in all these there is a splitting between the α and β carbon atoms.

Embden, Salomon and Schmidt now suggested that the NH_2 group might be the cause, so they took the corresponding fatty acids.

iso butylacetic acid,

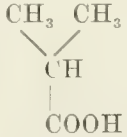


and iso valeric acid,



and found that the iso butylacetic acid, corresponding to leucin, formed no aceton, whereas the iso valeric acid, corresponding to amid valeric acid, was a strong aceton builder, just an opposite effect from leucin and amido valeric acid. The last follows the same law as the splitting up of the chain, as Knoop found, namely, there was a cleaverage between the α and the β carbon atom.

If this view is applied to iso butylacetic acid, which in structure is like iso valeric acid, namely, that there is a splitting between the α and β carbon atoms, then iso butyric acid,

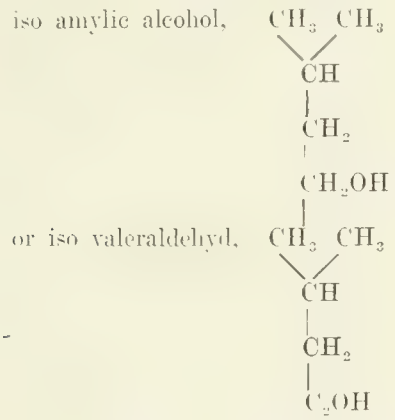


should be formed, and, if this is so, then this acid should give no aceton, which they found to be the case. The three homologs, iso butyric acid, iso valeric acid, and iso butylacetic acid, as regards their aceton building properties, behave exactly as do the aromatic fatty acids in their side

chains, and Knoop's experiments hold good for the aliphatic fatty acids. Why leucin and amido iso valeric acid are split up differently from their corresponding fatty acids is explained by Embden and his co-workers:

Leucin and amido iso valeric acid are changed by a splitting up of the carboxyl group into substances with one less carbon atom.

Whether the leucin is changed to iso valeric acid by oxidation of its carbon atom after denitrification, or whether amido iso valeric acid is converted into iso butyric acid in the same way, or if only the precursors of these fatty acids are formed—for instance,

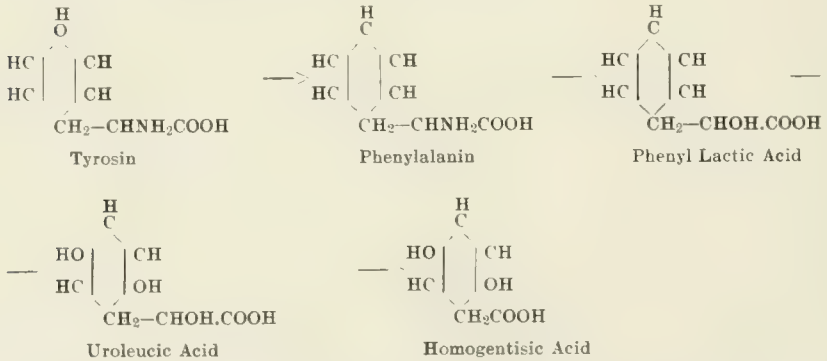


from leucin—they do not say. Whichever way they are split up, their further cleavage after splitting of carboxyl group follows the same law as that of fatty acids. Leucin, according to this, would be split up just the same as iso valeric acid and the amido iso butyric acid like iso butyric acid. Therefore, leucin perfusion gives rise to aceton as does perfusion with iso valeric acid, and amido iso valeric acid does not, as is the case with iso butyric acid.

Magnus Levy showed that oxybutyric acid can form aceton in the animal organism, and Embden et al. found this to be the case in perfusion of liver, where almost ten times as much aceton was formed as is seen with perfusion of normal blood. Perfusion of muscle led to negative results. Since oxybutyric acid is probably formed by oxidation from butyric acid, they tried the latter and found that aceton was formed, doubtless with the intermediary formation of oxybutyric acid.

They studied perfusion with l-tyrosin, $HO\text{-}C_6H_4\text{-}CH_2\text{-}CHNH_2\text{-}COOH$, and racemic phenylalanin, $C_6H_5\text{-}CH_2\text{-}CHNH_2\text{-}COOH$, which it will be seen, are amido acids united to the benzene radical, and found that both produced aceton. The phenylalanin minus the amido group or β phenyl a lactic acid, $C_6H_5\text{-}CH_2\text{-}CHOH\text{-}COOH$, also produced aceton, though not to such a marked degree as tyrosin and phenylalanin. As in

these substances the aromatic nucleus is burned up in the body, the next point to prove was whether aromatic substances themselves could produce aceton, in other words, if the aceton formation is in any way connected with the combustion of the aromatic nucleus. They selected β phenyl β lactic acid, C_6H_5-CHOH-CH_2-COOH, phenylacetic acid, C_6H_5-CH_2-COOH, and cinnamic acid, C_6H_5-CH:CH-COOH, and found that all of these failed utterly to produce aceton, and they conclude that the source of aceton in tyrosin, phenylalanin, and phenyl α lactic acid is the easily attacked aromatic nucleus, while in β phenyl β lactic acid, phenylacetic acid, and cinnamic acid, as the nucleus is not oxidized, there is consequently no aceton. How the benzene ring is utilized to form aceton is not certain, but, according to Neubauer and Falta, phenylalanin is changed to uroleucic acid, then to homogentisic acid, and in this acid the benzene ring is attacked, and Embden et al. confirmed this by finding aceton increased by perfusion with the latter.



Embden, Salomon, and Schmidt make several conclusions, which, briefly put, are that the aliphatic monamido monobasic acids are broken down to a body containing one less carbon atom, probably the corresponding fatty acid, and as such they are burned up as fatty acids are, namely, by oxidation in the β position.

Without detailing the important contributions of Baer and Blum,[31] it may briefly be stated that after a very careful study of the result of giving fatty acids to diabetics, they conclude that branched fatty acids, with an even group of carbon atoms, are productive of β oxybutyric acid and with this, aceton, whereas fatty acids, with 3 or 5 carbon atoms in a straight chain are not; and especially productive of oxybutyric acid are those acids where oxidation takes place in the β position. Of course, when β oxybutyric acid was increased, aceton was increased also, lending

31. Arch. f. exper. Path. u. Pharmakol., 1906-7, lv, 89, and lvi, 92.

additional weight, if more were necessary, to the belief that aceton is an end-product of β-oxybutyric oxidation.

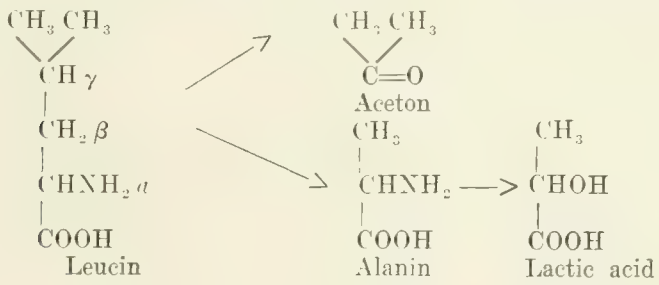
In order to see if Embden, Salomon and Schmidt's observations on the perfused liver could be repeated by feeding the same substances, Borchardt and Lange[14] experimented on themselves. They put themselves on a carbohydrate free diet and produced constant aceton output, then they gave themselves amido acids, beginning with glycocoll, CH_2NH_2-COOH, and found no increase, but rather a diminution of aceton. Embden[28] had found the same thing with perfusion. With alanin, CH_3-$CHNH_2COOH$, there was a diminution of aceton, confirming previous observations of Embden et al. The cause of this is due to the conversion of alanin into lactic acid by denitrification,

$$\begin{array}{ll} CH_3 & CH_3 \\ | & \\ CHNH_2 & CHOH \quad \text{Lactic acid} \\ | & \\ COOH & COOH \end{array}$$

and this acid has been found by Neuberg and Langstein after feeding rabbits on alanin and also by Embden in his perfusion experiments. Studies by Satta have shown that lactic acid is an antiketoplastic body, so that the explanation seems to be a suitable one.

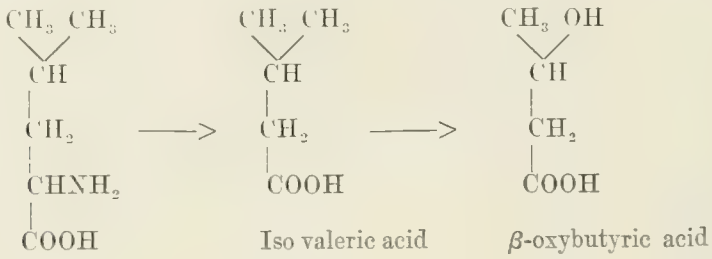
The next acid to be experimented with by Borchardt and Lange was the amid of the dibasic aspartic acid, asparagin, $COOH.CHNH_2.CH_2$-$CONH_2$. They found this produced a decided diminishing effect on the excretion of aceton bodies, as did Embden, Salomon and Schmidt. Glutaminic acid, a amido glutaric acid, $COOH$-CH_2-CH_2CHNH_2-$COOH$, was found in perfusion to have no influence on aceton bodies and on feeding they found the same thing.

Leucin caused a marked increase in aceton, as Embden found. Embden, Salomon and Schmidt believe this occurs by splitting of molecule between β and γ carbon atoms, giving rise to aceton directly. In this case lactic acid should be formed from lower half, but Embden could not find this, nor could Borchardt and Lange.



It seems to me that were the leucin split up in this way the aceton should be decreased, as lactic acid is an antiketoplastic substance (Satta).

Baer and Blum give another explanation, according to the law that fatty acid derivatives with substitution at the *a* carbon atom behave exactly as does the next lower fatty acid; in this way iso valeric acid would be formed, which, in turn, by substituting an hydroxyl group for a methyl group would be converted into β-oxybutyric acid, as shown by the following formula:



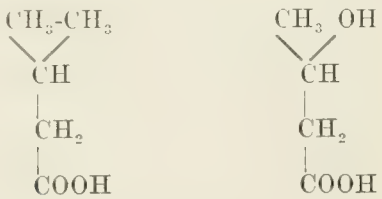
Leucin Iso valeric acid β-oxybutyric acid

This seems to be the way of cleavage, for if it were split up over alanin and lactic acid sugar should be formed, as the last two substances are known to be conspicuous sugar formers, while leucin is known to have no effect on sugar output.

Borchardt and Lange conclude their interesting paper with the following conclusions regarding the cleavage of fatty, oxy and amido fatty acids:

1. In the formation of aceton bodies, oxybutyric acid is first formed, from which by further oxidation diacetic acid is formed, and, finally, by cleavage of carboxyl group aceton is produced (Minkowski).

2. Amido and methyl groups united to a carbon atom can be substituted by a hydroxyl radical; in branched fatty acids, with two terminal methyl groups, one of the methyl groups is substituted by a hydroxyl, for instance,



iso valeric acid β-oxybutyric-acid

3. *a*-oxy and *a*-amido acids behave as do the next lower fatty acids (*a* amido valeric acid like butyric acid, leucin like iso valeric acid).

4. Unbranched fatty acids with an even number of carbon atoms and with the group CH_2-CH_2-CH_2-COOH can be oxidized in the β position, split in the same position and again oxidized in β position, until they are broken down to β-oxybutyric acid.

Branched fatty acids with uneven carbon atoms are probably split in same way and lead to oxybutyric acid formation.

MODERN THEORIES AS TO CAUSE OF ACIDOSIS AND COMA.

Lépine[32] says that acidosis does not explain all the symptoms of coma, especially the pulse and dyspnea, and quotes Klemperer and von Noorden as holding the view that coma is due to an unknown toxin in the blood. Sternberg (quoted by Lépine) has shown that after ingestion of β amido butyric acid there is acceleration of the pulse and respiration and appearances of coma, and that one gram per kilogram of body weight is fatal. β-oxybutyric acid, to the extent of nine grams, on the other hand, has been injected into a dog weighing 7 kg. without any ill effects, and he suggests that β amido butyric acid may produce β-oxybutyric acid with splitting off of NH_3.

CH_3CHNH_2-CH_2-COOH + H_2O = CH_3.CHOH.CH_2.COOH + NH_3.

One explanation for the increase of NH_3 seen in diabetic coma is thus offered, and the second source would be the protein tissue, as will soon be shown.

Lépine thinks the amido butyric acid is derived from the nitril of amido butyric acid as follows:

CH_3-$CHNH_2$.CH_2.CN + $2H_2O$ = CH_3-$CHNH_2$-CH_2-COOH + NH_3.

Gautier (quoted by Lépine) has found that albuminous substances contain complex nitrils. Some are innocuous, but by the action of certain reagents nitrils can become very poisonous, as cinnamic nitril, which produces symptoms analogous to hydrocyanic acid poisoning. Lépine's theory based on Gautier's observations and his own work is, that not the β amido butyric acid or β-oxybutyric acid, but rather the complex nitrils of protein tissue are the cause of acidosis. In support of this view he concurs with Fiquet in recommending phenol in diabetes, as combinations of this with nitrils renders them innocuous, and believes it should be tried. This very suggestive work of Lépine does not seem to have been followed up, and, despite the fact that only *a* amido acids are seen after protein cleavage, there are a great many things in favor of his theory, which require further study to be put on a firm basis.

32. Semaine méd., 1900, p. 399.
33. Deutsch. med. Wchnschr., 1900, p. 757.

Spitzer[33] does not lay much stress on the β-oxybutyric acid theory of acidosis, as even diabetics can use up a good deal of oxybutyric acid, and in pancreatic diabetes no acidosis can be produced with oxybutyric acid. He agrees with Magnus Levy that the poverty of the organism in fixed alkalies is not the cause, as Naunyn held. Spitzer holds to the view that carbohydrates are to be regarded as the underlying condition, as cases of diabetes are constantly met with where acidosis appears on going over to a carbohydrate free diet. Here no increase of acid is ingested, the main reason for the appearance of coma being that in breaking down of carbohydrates, substances are formed which, uniting with the decomposition products of protein and fat, render easy the oxidation to CO_2 and H_2O. Absence of these substances, as is seen in carbohydrate hunger and in diabetes, leads to acidosis, as the oxidative power of the organism is not enough in itself.

The disturbance of the carbohydrate metabolism, preventing oxidation of protein and fatty decomposition products, Spitzer believes to be due to psychic or physical trauma, for such cases are often seen leading to rapid death from acidosis. Again, cases of mild diabetes have been operated on, with rapidly ensuing coma after narcosis, and Spitzer does not agree with Naunyn that the narcosis has caused rapid tissue breakdown, explaining the acidosis, but thinks the time is too short for such an extensive destruction to take place, and that psychic shock best explains it. Spitzer reports a case of mild diabetes with absence of acidosis even after fasting, but which succumbed to coma after fracture of the clavicle. In this instance there was no oxybutyric acid, in fact, numerous cases have been published of coma with high NH_3 output, yet with no aceton or diacetic acid (Rumpf, Münzer, Strauss, quoted by Spitzer). Spitzer suggests that, as after the celebrated Piqûre, the liver is "frightened," shakes out all its glycogen and loses the power to hold back sugar coming to it.

Grube[34] has concisely summarized the four main theories of diabetic coma, and I shall briefly reproduce his remarks:

1. *Theory of Coma on Basis of Aceton Poisoning.*—He does not believe in this for three reasons: (a) aceton can be excreted for years with no symptoms of coma, (b) in a few cases of coma aceton has been absent, (c) after ingestion of aceton there is no intoxication, and even with large doses the symptoms are not those of diabetic coma.

2. *Uremic Theory.*—There is a wide difference between the two disorders, as uremia is never seen with healthy kidneys and coma very often is.

34. Arch. f. exper. Path. u. Pharmakol., 1900, xliv, 349.

3. *β-oxybutyric Acid Theory* (Stadelmann, Naunyn, Magnus Levy).—Stadelmann suggested that the symptoms of coma were due to increased acid formation, and believed that crotonic acid was the cause, but this was soon controverted by Minkowski and Külz, who found not crotonic acid, but β-oxybutyric acid. This theory, that of a β-oxybutyric acid intoxication, seems to be somewhat weakened by the studies of Walther, who produced symptoms similar to coma with hydrochloric acid and phosphoric acid. Furthermore, other acids, diacetic acid, lactic acid, and fatty acids are seen, and have each been held responsible for coma, though Magnus Levy claims they play no quantitative rôle.

4. *Unknown Toxin Theory* (Klemperer).—This theory holds that both coma and β-oxybutyric acid are caused by these toxins.

Grube seems to incline to this view (see Lépine), and experimented on cats with β amido butyric acid, producing coma in all of his twenty experiments, and, according to the amount of acid ingested, the severity of the coma was influenced, together with appearance of aceton and sugar. He has thus confirmed Sternberg's work, and in this he must be classed with Lépine, but he doubts whether β amido butyric acid is formed in the diabetic organism.

Magnus Levy[35] repeats in this work his former assertion that diabetic coma is an acid intoxication. The acidosis is so extreme in coma that the acid withdraws from the blood and tissue the alkalies of the carbonates and phosphates and destroys the vital groups of protein molecule, especially the proteid of the ganglia body cells. As a proof of this loss of oxidation power of organism, one sees in diabetic coma diminution of alkalies of blood and appearance of large quantities of β-oxybutyric acid in the blood.

Busse[36] agrees with the above, and states that in autopsies made on diabetics he has found parenchymatous degeneration of kidney and heart, and sometimes of liver, which he attributes to the action of β-oxybutyric acid.

Orlowski[37] studied the alkalinity of blood in diabetes and found it to be invariably lowered, due, as he says, to β-oxybutyric acid, which he regards as the underlying cause of coma, aceton, and diacetic acid being merely secondary. He gives a rather broad definition of acid intoxication, inasmuch as he regards the diagnosis as certain only when β-oxybutyric acid is found in the blood and urine, and when there is improvement in the condition with the use of alkalies. I should take exception

35. Id., 1901, xlv, 389.
36. München. med. Wchnschr., 1901, p. 1404.
37. Centralbl. f. Stoffwechsel., 1902, p. 31.

to the last statement, and I believe many clinicians would agree with me that many cases of coma do not recover under the use of sodium bicarbonate or alkalies in general, but they are nevertheless cases of coma. I recall a case, studied with Dr. Musser, where coma was prognosed several days before its onset, alkaline therapy was instituted intravenously, subcutaneously, per os, and by enteroclysis, despite which the patient gradually grew worse and died with the typical symptoms of diabetic coma. To make a diagnosis of such a severe condition on a therapeutic test seems to me to be unwise.

That aceton can cause autointoxication is shown in the report of two cases by Pettera,[38] but he specifically states that there was no resemblance between diabetic acidosis and his cases, nor was there ever found any sugar or β-oxybutyric acid. The condition is hardly to be confused with diabetic coma, although his report contradicts statements made by other observers that aceton is not toxic (Grube[34]). To these cases may be arrayed a third, reported by Palma,[39] of three attacks of autointoxication, when the only underlying condition was great increase of aceton in urine and probably in the breath, although Folin, in his Harvey lecture of 1908, said that the peculiar aceton odor of breath is not caused by aceton in the great majority of the cases.

Eppinger, in 1906, published two important papers dealing with acidosis which I consider worthy of detailed review. In the first contribution,[40] he reviews some of the previous work on this subject, starting with Salkowski's work on the utilization of tannin by herbivora. Tannin, it will be remembered, is amido ethyl sulphonic acid, $C_2H_7NSO_3$, or $CH_2NH_2CH_2SO_2OH$, and Salkowski found that herbivora used up tannin with the liberation of H_2SO_4, and that if the feeding of tannin were continued for any length of time the animals invariably died. This he believed due to the withdrawal of alkalies from the body by the acid, and found the bases of the urine increased after tannin. The same thing was noted after ingestion of H_2SO_4. Gäthgen, in attempting to corroborate this, fed tannin to dogs without any success, and ascribes the difference between his work and Salkowski's to the difference in chemical constitution of herbivora and carnivora.

Walther, according to Eppinger, laid the foundation of our modern view of acidosis, and found that if an acid which is not oxidized were introduced into rabbits, the CO_2 of the blood diminishes very rapidly, and when the height of the poisoning is reached it disappears entirely.

38. Prag. med. Wchnschr., 1905, p. 199.
39. Wien. klin. Wchnschr., 1906, No. 5.
40. Wien. klin. Wchnschr., 1906, No. 5.

He believes this is due to the combination of blood alkalies with the acid and, the blood being no longer able to extract the poisonous CO_2 from the tissues, there is an accumulation, and the animal succumbs to what he graphically terms "internal suffocation." He substantiates this view by noticing the remarkable beneficial effect of intravenous injection of alkalies in these cases. In dogs, on the contrary, he did not find this diminution of carbonic acid in the blood, and, as the fixed alkalies remain unchanged, he thinks the organism of a carnivorous animal has other means of protection against acid intoxication. This protection he ascribes to NH_3, which he found increased in the urine, and which he believes prevents the export of fixed alkalies from the blood.

Eppinger believes that the constitutional difference between carnivora and herbivora, suggested by Gäthgen, is not the fundamental reason for such divergence of results, but thinks the discrepancy is rather one of degree. Winterberg and Limbeck have shown that by gradually increasing the dose of the acid they could increase the usual lethal dose for a rabbit, which was accompanied by a slight increase of ammonia, and Spiro later showed that dogs could be killed by acid intoxication. These experiments tend to render the view of a "constitutional difference" in the two species untenable, but the question naturally arises, If in carnivora the ammonia is the main protection against acidosis, where is it stored up, for we know protein tissue is the only source? As rabbits have practically no ammonia for protection, Eppinger thinks that if nitrogenous bodies derived from protein decomposition are fed to rabbits they should save the animals from a fatal acid poisoning, and, furthermore, that their resistance should be accompanied with an increased output of ammonia in the urine.

Working on this hypothesis, he gave rabbits the fatal dose of hydrochloric acid, and followed immediately with amido acids, finding that glycocoll ($CH_2NH_2.COOH$), alanin (CH_3-$CHNH_2$-$COOH$) and aspartic acid ($COOH.CHNH_2.CH_2COOH$) all prevented acidosis. Two reasons are suggested: Either that this action was due to combination of the amido acid forming glycocoll hydrochlorate, which is excreted as such, or that oxidation takes place, setting ammonia free, which uniting with the acid renders it innocuous.

That the first was not the case was proved by the fact that the rabbit rapidly succumbed to an aliquot amount of glycocoll hydrochlorate. If the second procedure takes place, there should be more NH_3 found in the urine, which he showed to be the case. That the amount of ammonia derived from the amido acids is not enough to neutralize the acid, and that fixed alkalies are a factor, he proved by finding an increase of potas-

sium and sodium in the urine. Further confirmation of this is offered, as he found there was a slight withdrawal of alkalies from the blood.

The acid amids, foramid, $CHO.NH_2$, and acetamid, CH_3-$CONH_2$-exert no such effect as would be expected, as Schultzer and Nencki[41] found that the acid amids are not changed in the body.

Having established that amido acids, the lowest cleavage products of protein decomposition, offer immunity against acidosis, Eppinger tried the effect of polypeptids—glyzylglycin and leucylglycin—which only retarded the death of the rabbit, while with pepton and gelatin the animal died very promptly from acidosis. This seems to prove to Eppinger's mind that the kind of food, and the way such food is utilized by the organism, is the primary condition in acidosis, and he thinks that diabetes may be an expression of disturbed proteid as well as of disturbed carbohydrate metabolism.

In a second paper Eppinger,[42] after reviewing the main substance of his first article, thinks that his experiments have some practical value in diabetes. After showing that the body derives the protective ammonia mainly from the food, he believes that the reason the organism succumbs to diabetic acidosis is because there is not enough protein food given to furnish the required amount of ammonia, and as the body is unable to furnish fixed alkalies for any length of time, acid intoxication is bound to follow. The gastrointestinal disturbances preceding may be due to a defective breaking down of protein, with the necessarily resulting diminution of ammonia. In coma, therefore, Eppinger recommends the use of urea subcutaneously, and amido acids per os and per rectum, although he is inclined to Ebstein's view, namely, that diabetes is more a disease of entire protoplasm which prevents the organism from using even amido acids (Mohr). Despite the apparent absurdity of giving a person substances which are not utilized, Eppinger, nevertheless, recommends trying amido acids, as he considers the treatment of coma futile in any case, and the amido acids can do no harm.

As Folin points out, "the alkali therapy must, after all, furnish the final practical proof of the validity of the acid intoxication theory," and this is true, to a certain extent, but it must be remembered that not all cases of diabetic coma are comparable. Some are seen early and some late in the course of the disease, and this must vitiate to a certain extent the results obtained with the alkali treatment. Remembering the work of Busse,[36] the destructive changes wrought by β-oxybutyric acid must be borne in mind, and if, as Ebstein (quoted by Eppinger) states, diabetes

41. Hammarsten. Physiol. Chem., 5th edition, p. 546.
42. Ztschr. f. exper. Path. p. Therap., 1906, p. 530.

is a disease of the entire body protoplasm, it will be seen that when destructive changes take place in the body just how futile any treatment must be. That early cases of coma are benefited by alkaline remedies there can be no doubt, and even in the advanced cases improvement and sometimes postponement of lethal termination have been observed.

β-oxybutyric acid seems at present to be the cause of acidosis. Why this acid is formed is as much a riddle as ever before, and the glib answer that the body has, in a measure, lost its oxidative power is but a confession of our ignorance of the subject in question. All we know is that β-oxybutyric acid is seen in advanced diabetes, is seen in coma, has been found in excessive amounts in the organ tissues of diabetics dying in coma (Magnus Levy), and appears to be the cause of diabetic acidosis. The ever-recurring "But why" must be left for future investigators to answer. Theories there are, and many, but theories without proof remain but theories, and are to be adjudged as such.

SUMMARY AND CONCLUSIONS.

Together with a clear understanding of diabetic acidosis must be brought a knowledge of normal physiologic facts, which the foregoing, dealing only with the important publications of the past eight years, hardly touched on. It will be remembered that during the breaking down of carbohydrate, fats, and proteins numerous products of an acid nature are normally formed. These acid bodies are for the most part carbon-dioxid, sulphuric, hydrochloric and phosphoric acids, besides uric acid, fatty acids, and occasionally other acids. The carbon-dioxid is mostly excreted by the lungs and, in part, by the skin, while the sulphuric acid and other acids are excreted in the urine. These acids are, however, never found as such, but are combined with the body alkalies, forming salts. To neutralize these acids, the body must be deprived of its alkalies, and this loss is covered mostly by the alkalies contained in the ingested food. Besides these, however, the body contains a certain amount of "reserve alkali" which is found in the protoplasm of the body cells as potassium. The alkalies ingested with the food are found as sodium salts in the circulating blood, and to their presence is due the alkalinity of the blood. Besides these combinations of alkalies with inorganic acids, sodium phosphate and sodium carbonate are present, and these also help in preserving the blood alkalinity. The amount of these alkaline bodies is not great; 100 grams of blood contain a quantity equal to only about 180 mgr. of NaOH (v. Noorden).

If alkalies are being constantly withdrawn from the body by the intermediary products of tissue decomposition, it will readily be seen that

abnormal sapping of the alkali reserve can be accomplished by lessening the ingestion of alkalies in the food or by increasing the amount of the acid in the body. We have seen from Eppinger's work what fatal effect the administration of acids has on rabbits. Not only are there symptoms of acid intoxication, rapid respiration and increased pulse rate with convulsions, coma and death, but there is also an increase of the bases of the urine with a diminution of the blood alkalinity and of the carbon-dioxid from the lungs, indicating withdrawal from the body of alkalies. It will also be recalled what prompt beneficial effects follow the administration of alkalies. In dogs we have learned that, besides these labile and fixed alkalies of the body, there is a further protection offered by ammonia which is, for the most part, derived from the protein food. These teachings of Walther, Eppinger and others we have transferred to man, and we know that, thanks to our carniverous tastes, we are able to produce ammonia in such enormous amounts that our protection against even high acid values in the body is assured. Even under pathologic conditions, such as diabetic acidosis, v. Noorden reports that an output of ammonia from 4 to 6 grams a day is not at all uncommon, and Stadelmann once found 12 grams. When it is recalled that the normal output is between .3 and .7 grams, depending, of course, on the ingested food, and that each gram above that accounted for by the food corresponds to 6.12 grams β-oxybutyric acid, it will be seen what a great protection the ammonia is to us and what great quantities of acid it can neutralize.

The name acidosis was coined by Naunyn to cover the symptom-complex of the presence of increased acid in the body, and is, of course, always a sign of disturbed metabolism. This increase of acid can occur in two ways, deficient elimination and increased production. The first is relatively unimportant and never produces the symptoms of acid poisoning. The second is of prime importance, and may be associated with the former, when it is of double significance. The three acids with which this paper deals are β-oxybutyric acid, diacetic acid and aceton, called, for convenience, aceton bodies (Geelmuyden). Of these three, only aceton is seen normally in the urine, although some modern writers contend that this is not so, that aceton is never preformed, but is really diacetic acid. Until this matter is cleared up I prefer to regard the aceton determined by our qualitative tests, as aceton. That aceton is a product of normal metabolism can not be denied, and, though there is some doubt as to its toxicity, I believe it has been pretty definitely shown that aceton does not produce acidosis (Grube, Satta). Diacetic acid is also readily oxidized when given to healthy individuals, and we have seen from Lépine's experiments what large quantities of β-oxybutyric

acid a dog can take care of without showing symptoms of acid intoxication. What, then, causes the symptoms of acidosis and coma if the aceton bodies are so innocuous? The correct answer I believe to be is increase of acid in the organism. This acid might just as well be sulphuric acid or any other acid, if such could be formed in equally large amounts as β-oxybutyric acid, but it so happens that in diabetes β-oxybutyric acid is the cause. It must be insisted on that β-oxybutyric acid possesses no individual toxic properties not shown by other acids, but the deleterious effects ascribed to it are due to its purely acid character and, owing to its acid nature, it has the power of extracting from the body its alkalin supply and in this way producing acidosis.

The source of these aceton bodies now seems to be practically settled. That the principal source is from fat, especially fat containing fatty acids, can not be denied after the abundant proof offered. That the aceton bodies can be derived from protein has been shown by Blumenthal and Neuberg, also by Orgler, *in vitro,* but conclusive proof has been brought by Embden, Salomon and Schmidt, after finding increase of aceton after perfusion with leucin. That the formation of aceton from albumin takes place over fatty acids after denitrifying the amido acids is also a well-established fact, so that there should be no further controversy about the question whether aceton is derived from fat or proteid. Carbohydrates are not the source of aceton bodies.

The place of formation of aceton bodies is probably in the liver (Embden and Kalberlah, Embden, Salomon and Schmidt), although there are some supporters of the intestinal origin, especially J. Müller. Lüthje has found that acetonuria is not diminished by the use of laxatives and intestinal antiseptics, and v. Noorden has noticed even an increase following such measures, so this seems to disprove the enterogenous theory of aceton formation (Satta).

A CASE OF INFECTION WITH SCHISTOSOMA JAPONICUM IN A FILIPINO.

EUGENE R. WHITMORE, M.D.

MANILA, P. I.

For over twenty years physicians practicing in Japan have observed, in certain provinces, a peculiar cirrhosis of the liver with diarrhea, fever, anemia, ascites and edema. In some of these cases ova of an unknown parasite have been observed in the liver and other organs.

In the latter half of 1904 Katsurada[1] described ova found in the feces of patients and in the tissues of men and cats. He also described the adult worms as found in the portal and mesenteric veins in cats, and called the parasite *Schistosoma japonicum*.

Early in 1905 Catto[2] reported a case in a Chinaman from the province of Fukien. He described minutely the ova found in the tissues, and the parent worms found at the bifurcation of the smaller mesenteric vessels. Catto called the parasite *Schistosoma cattoi* (Blanchard). This case was previously published as one of coccidiosis in man, and again as one of infection with a new trematode.

Logan[3] has reported three cases in Chinese.

In January, 1906, Woolley[4] reported a case of infection with *Schistosoma japonicum* in a native Filipino, and so far as I know this was the first case reported from the Philippine Islands.

My case is that of a Filipino scout about 30 years old, who had been in the service of the United States about eight years.

Patient.—The man was born on the Island of Samar, was enlisted at Tagabian, Samar, and his company has been stationed there for some time. He had never been away from the Philippine Islands, and most of his life had been spent on the Island of Samar.

History.—The man was on furlough and entered the Division Hospital, Manila, P. I., at 10:00 a. m., January 16, 1908, in the service of Captain Ruffner, to whom I am indebted for all the clinical notes. The man complained of nothing but weakness and there was nothing in his appearance or on examination that would indicate that he was seriously ill. His temperature was 98.6 F., and his pulse 120. At 1:00 a. m. on January 17, the man began having diarrhea and severe

1. Jour. Trop. Med., London, 1905, viii, 108.
2. Jour. Trop. Med., London, 1905, viii, 70.
3. Jour. Trop. Med., London, 1906, ix, 294.
4. Philippine Jour. of Sci., January, 1906, p. 83.

abdominal cramps. He passed quickly into collapse and died at 6:45 a. m., January 17.

Autopsy.—As there was considerable cholera in Manila at the time we had at the autopsy to look out for cholera and beriberi.

General Appearance.—The body was that of a normally developed Filipino. There was no edema and no excess of fat was noted on opening the abdomen or about the abdominal viscera. There was no ascites.

Liver.—The liver appeared slightly enlarged, but about normal in color. The smooth surface was studded over with numerous yellowish spots from 1 to 1.5 mm. in diameter. The liver cut with increased resistance, the capsule was thickened, and the connective tissue about the gall bladder and along the larger bile ducts was markedly increased in amount, and was edematous and bile stained. The same yellowish nodules seen on the surface were dotted throughout the liver substance. Crushing the nodules showed them to contain a number of brown ova. The gall bladder was distended with thick bile, but the passage into the duodenum was free. Microscopically these miliary nodules have a caseous center and a fibrous margin with rather distinct line of demarcation from the liver tissue. They are usually connected with an area showing new formation of bile ducts. The general picture of the liver is that of a hypertrophic cirrhosis with new formation of bile ducts. Ova were found in some of the nodules; in masses in new formed connective tissue which shows new formation of bile ducts; and singly in the small vessels among the liver cells. In the latter position there is often no evidence of new formed connective tissue.

Intestines.—In the upper part of the rectum was an area, two inches long and extending half way around the rectum on the mesenteric side, in which the mucosa was swollen, red, and very slightly and superficially eroded. Otherwise the intestine and its contents appeared normal, except for a few uncinaria in the upper part of the small intestine. Cultures for comma bacillus were negative.

Microscopically the mucosa of the large intestine is normal, except for slight superficial erosion. The submucosa is thickened and there is considerable round celled infiltration. The muscularis is normal as is the subperitoneal tissue. Ova are found in fair numbers in the submucosa in areas of round celled infiltration. No ova are found in the other layers. Microscopically the small intestine shows nothing abnormal.

Kidneys and Suprarenals.—There was a yellowish nodule 2 mm. in diameter, 3 mm. from the base of one of the pyramids of the right kidney. Microscopically, this nodule is composed of fibrous tissue with a few remains of the kidney tubules. Otherwise the kidneys appear normal. No ova are found in this nodule or any where in the kidney tissue. There were two yellowish nodules, 1 mm. in diameter, bulging above the surface of the right suprarenal gland. Microscopically, both of these nodules are composed of fibrous tissue. Otherwise the suprarenals appear normal. No ova are found in these nodules or anywhere in the suprarenals.

Lungs.—The lungs showed nothing but slight congestion. Microscopically, there is considerable round-celled infiltration in the alveolar walls, and some ova are found lying singly in the areas of infiltration.

The brain, thyroid, pancreas, bladder and prostate appear normal.

The spleen was somewhat enlarged, dark colored and bloody.

The mesentery showed nothing. The vessels were carefully slit up but no worms were found, and none have been found in sections of the mesentery.

Heart.—The heart was pale and flabby and I was inclined to think that the man had died of beriberi, but sections from the tibial nerves showed nothing abnormal. Katsurada says: "A certain number of the patients die of extreme weakness," and that is all that I can find to account for the death in this case.

It is possible that the worms form a toxin, as suggested by Katsurada, and that this toxin had something to do with the sudden death in this case.

Bacteriologic Examination.—Sections from the nodules in the liver, kidney and suprarenal were stained for tubercle bacilli and for spirochetes, but neither has been found. The ova found correspond in appearance and measurements with the ova of *Schistosoma japonicum.*

This case is of interest, as there were present practically no other parasites and no lesions other than those due to the schistosoma, and those lesions were of the nature of a fibrosis.

Whether or not the nodules in the kidney and suprarenals were caused in any way by the schistosoma I can not say. I find no mention of lesions in the kidneys or suprarenals, and Catto says: "The kidneys, suprarenals, . . . showed no signs of coarse disease."

THE TREATMENT OF GONOCOCCUS ARTHRITIS BY INJECTIONS OF DEAD GONOCOCCI, AND THE CLINICAL REACTION WHICH FOLLOWS THE INJECTIONS.*

E. E. IRONS, M.D.

CHICAGO.

[From the Memorial Institute for Infectious Diseases and the Presbyterian and Cook County Hospitals.]

Relatively few cases of gonococcus arthritis and periostitis treated by the subcutaneous injection of dead gonococci have so far been recorded. Cole and Meakins[1] reported fifteen cases of gonococcus arthritis in which inoculations were given and systematic observations made on the opsonic index. After a careful study of the cases they concluded that inoculation of dead gonococci appeared to have a decided value in favorably influencing the course of the disease.

This paper is based on the study of forty cases of gonococcus infection, including thirty-one cases of arthritis.[2] The diagnosis of each case included in the series was determined beyond any reasonable doubt, from the clinical history and course as well as from the associated genital infection. In all the cases in any way obscure the diagnosis was confirmed by the isolation of the gonococcus from the joints, and in two cases from the blood.

In fifteen of the forty cases studied the opsonic index was systematically determined. The initial indices were usually low, with rises following injections of dead gonococci. Aside from the injections of dead cocci, other factors, such as massage of the prostate and massage of affected joints, were found to raise the index. This has been observed also by Wright and others. Furthermore, spontaneous fluctuations in the index occurred in untreated cases of arthritis, due possibly to trauma or movement of an acutely inflamed joint.

Taking these facts into consideration, and bearing in mind that the opsonic curve is the expression of only one of the many changes which

*Read before the Chicago Pathological Society, March 9, 1908.

1. Treatment of Gonorrheal Arthritis by Vaccines, Bull. Johns Hopkins Hosp., xviii, No. 1905, June, 1907.

2. The histories of these cases, with details, will be found in a more complete paper to appear in the Journal of Infectious Diseases, vol. v, No. 3.

may take place in the body fluids in the course of infections, and that for some time at least inoculation therapy will depend for its control largely on clinical observation, it seemed advisable in the present work to use the clinical manifestations as the guide to the size and interval of the injections.

The lesions that occur in the course of generalized gonococcus infections present certain characteristics that render these cases peculiarly favorable for the observation of the clinical effects of therapeutic injections of dead cultures of gonococci. The course, even in the acute cases, extends over a number of days at least, and much more often over weeks and months. The fever and other constitutional disturbances are relatively less than in other infections of corresponding extent in the body, and the lesions, involving chiefly the joints, tendons and periosteum, are readily accessible to examination and careful observation.

In order that the inoculation treatment may have a general practical clinical value in the event of the experimental results receiving further confirmation, the dead cultures must be prepared, at least for the present, from heterologous organisms, for in many cases it is not only impracticable but impossible to obtain the so-called homologous strain.

Accordingly, for the purpose of uniformity, all the cases in this series were treated with cultures obtained from other sources than the patients in question. The cultures of the gonococcus used were isolated from various lesions, including urethritis and arthritis.

To obviate, in part at least, certain objections to the use of heterologous organisms, some of the material was prepared from two and sometimes three strains of the gonococcus. In many cases, however, but one strain was used. In a clinical study of this nature it is a matter of extreme difficulty adequately to compare the results with different kinds of killed gonococci. The general impression is that the immunizing responses were as good when one strain was used as when two or three were combined.

In the early cases treated, from 20,000,000 to 50,000,000 organisms were given at a dose. A dose of this size usually produced a rise in the opsonic index, but often no coincident change occurred in the clinical course of the disease. It seemed advisable to increase the dose, and accordingly in the later work from 100,000,000 to 1,000,000,000 organisms were given. The intervals between injections varied from three to seven days.

THE GONOCOCCUS REACTION.

In following the clinical course of the cases under inoculation treatment daily records of both subjective and objective conditions were made in so far as this was possible. It soon became evident that the course of events following the inoculation was not always one of progressive improvement. When seen twenty-four hours after the injection, patients repeatedly complained that the joint pains were worse rather than better. At this period of the work the dose used was relatively small, and the exacerbation of the symptoms did not occur regularly after each injection, so that for a time the phenomenon was regarded as an accidental coincidence. When the initial dose was increased, however, it was found that frequently the patients suffered from increased articular pain and tenderness after the first, often after the second, and occasionally after subsequent injections. Four cases in the same ward were given their first injections on the same day, the dose being 500,000,000. On the following day two of the cases showed only a slight increase in symptoms, with some rise in temperature and pain in the joints. In these two the arthritis was not extensive. In the other two cases in which the joint involvement was greater there was a decided exacerbation of symptoms. The involved joints became more acutely tender, and the fever which had previously not exceeded 99.5° F. rose to 101.5°, associated with general malaise. On the second day after the injections the acute symptoms had subsided and the patients felt about as well as before the injections were given. During the succeeding days improvement was more rapid than before the injections, so that the net gain of the first week of treatment was greater than that of the week preceding. In these cases the clinical picture after injection was most striking, and was highly suggestive of the phenomena observed in the tuberculin reaction.

The clinical records of the cases treated before this observation was made were now examined, and in those in which daily records had been made it appeared that the characteristic reaction had occurred, though by no means to so great a degree. In subsequent cases it usually has been possible so to graduate the dose of gonococci as to produce the reaction.

A typical gonococcus reaction is characterized by a rise in temperature, often only slight, an increase in pain and tenderness in the affected joints, with occasionally some increase in swelling and a variable degree of malaise. The symptoms follow the injection in from eight to twelve hours and commonly last twenty-four hours. In some instances there

is, too, a local reaction at the site of injection with tenderness, occasionally redness and some edema. These local signs commonly subside in twenty-four hours, though in three cases they persisted for three or four days. Great care has been taken throughout to insure surgical cleanliness in giving the injections. Moreover, in a number of normal individuals, to whom the same amount was given, no local reaction was noted. Leucocyte counts were made before and after the injections in a number of the gonococcus cases. Generally there was an increase in the leucocytes eighteen hours after the injections, though this was not constant. The degree of the reaction is influenced by several factors. After small doses (20,000,000 to 50,000,000 in the cases in question) the reaction was not marked, and in a number of the early cases treated it either did not occur or at least was overlooked. In one case (Case 1), however, in which a 50,000,000 dose was given, there was a reaction after each of the first three injections. When, beginning with a small dose, gradually increasing doses were employed, the reaction did not appear so typically, or was scarcely observable. When in a given case the same moderately large dose (300,000,000 to 500,000,000) was used for all the injections, the reactions became less with each injection. In four instances the reactions followed the initial dose only.

The most constant feature of the reaction is the increase in joint pain and tenderness. The temperature rise may be so slight as of itself to excite but little remark, especially in those cases in which there is a daily temperature of 99.5 to 100. In opsonic terminology the clinical reaction corresponds to the negative phase.

THE CLINICAL GONOCOCCUS REACTION IN DIAGNOSIS.

The frequency with which these clinical phenomena occurred suggested the possibility of utilizing the reaction in the diagnosis of obscure cases of arthritis in which the gonococcus was the suspected cause. The effects of the injection of dead gonococci into patients not suffering from gonococcus infection were accordingly studied. Eight adults in whom there was no history or sign of gonococcus infection were given subcutaneous injections of 500,000,000 dead gonococci. In none of these was there any local change other than that following an ordinary hypodermic puncture, and no fever or constitutional disturbance was observed. In a case of pyorrhea alveolaris with subsequent general infection, and painful swellings over the extremities, there was no increase of fever or local symptoms following the injection. A case of gout with

active joint involvement showed no local nor general change after a dose of 500,000,000. Leucocytes before injection 13,200, eighteen hours after injection 13,000; temperature normal throughout. A case of articular rheumatism showed no reaction after 500,000,000. There was no increase in leucocytes and the temperature chart showed no abnormal variations. A case of acute arthritis with pericarditis was thought possibly gonococcal in origin. There was no reaction after a dose of 500,000,000. Cultures from the blood and from a small amount of clear fluid aspirated from the knee remained sterile and the prostatic fluid contained no gonococci. The subsequent course was typical of acute rheumatic fever. In four other cases of acute and subacute articular rheumatism there was no reaction after injections of 500,000,000 cocci.

In a number of suspected gonococcus cases the reaction was of value in making an early diagnosis. A case of monarticular arthritis with effusion in the knee in which gonorrheal infection was denied was given an injection of 500,000,000. The evening temperature, which had previously reached only 100, rose to 101.8 and the joint pains increased. The knee was aspirated and the gonococcus isolated in pure culture from the fluid. A case of chronic arthritis which had resisted all treatment was given an injection. A slight rise in temperature, with increase in joint pains, followed. The prostatic fluid was found to contain gonococci, and the subsequent course was that of gonococcus arthritis. A patient who had suffered from extensive gonococcus arthritis had been bedridden for one year. There was practically no motion in the knees. After an injection of 500,000,000 the temperature, which for weeks had been normal, rose to 99.5, and the patient complained of malaise and increased pain in the joints. A patient with aortic aneurism who denied gonorrheal infection had been selected for control experimental inoculation. After an injection of 500,000,000 cocci the temperature, which had been uniformly normal, rose to 100 F. without any other apparent cause, returning to normal next day, with no subsequent rise. The prostate was examined and found to be large, somewhat tender, and the secretion contained numbers of leucocytes with typical intracellular gonococci.

CLINICAL RESULTS.

The clinical course of gonococcus infections is extremely variable. The large majority of the arthritic cases recover spontaneously, though frequently convalescence is long and tedious and the patient may be partially disabled for months. It is obviously fallacious to draw conclusions

as to the value of a treatment from the observations of isolated cases, and even when a number of cases of a disease so variable in its course as gonococcus arthritis are grouped together one must be very guarded in the conclusions as to the rôle of the treatment in effecting a cure. In summarizing the results the previous duration of the arthritis, its course in the days or weeks immediately preceding treatment, and the rapidity of subsequent improvement are of greater clinical weight than the mere statement of the time required to effect a cure.

In general the inoculations gave better visible results in the chronic than in the acute cases. In several acute cases improvement was apparently more rapid immediately following the injections than before. But while the acute cases terminated favorably and without undue delay, it must be recognized that they might have done so without treatment, though possibly less rapidly. The evidence in favor of the inoculation treatment is stronger in the subacute and chronic cases. In a number of cases in which after a typical acute course there remained more or less indolent swelling and tenderness in one or more joints, after the first injections the signs of the infection subsided rapidly. A patient (Case 26) had had a typical gonococcus arthritis for ten weeks. For six weeks before inoculation there had been no improvement in spite of treatment by rest and medicines. He was able to hobble around, but the pain in the lower dorsal region, sternoclavicular joint and in the heels rendered him unable to work. After the slight reaction following the first injection of 300,000,000 cocci his pain was much less, and after two more injections at intervals of four days he was discharged with no pain or other disability. When seen two months later he was still free from the trouble. A number of similar cases might be cited.

The treatment of several very chronic cases was undertaken. A patient (Case 10) had suffered for years from recurrent attacks of "rheumatism" in the region of the lumbar spine and in the heels. For seven months, with the exception of one month, he had been unable to work. At one time the heels, at another the back seemed to be worse. He had taken medicines of many kinds, electrotherapy, etc., without benefit. The prostate was large, tender, and the fluid obtained by massage contained typical gonococci. Inoculation of dead gonococci was followed by an increase in pain and tenderness for twenty-four hours, and then by improvement. After six injections during a period of one month his condition was very much improved and he was able to go to work. Each injection was followed by an exacerbation of local tenderness. The sub-

sequent improvement was much slower but progressive. The injections were continued with increased dosage, combined later with massage of the prostate, which was much less tender than at the outset of treatment. At the end of three months' treatment recovery was practically complete. The later injections were not followed by any observable clinical reaction.

In four of the thirty-one cases in the arthritic series the results were unsatisfactory. Two of these (Cases 18 and 22) were acute cases in which but two injections were given because of peculiar obstacles to treatment. These might reasonably be omitted from the series. Another early but extensive case (Case 2) received only small doses (50,000,000) for one month without improvement, and the treatment was discontinued. Massage of the prostate was substituted, with subsequent recovery after a slow convalescence.

The fourth case was that of a chronic alcoholic, who had also a chronic nephritis with considerable edema. In addition to his gonococcus arthritis, he had an otitis media and a dacryocystitis of a year's duration. Occasionally a slight reaction could be obtained after inoculation, with temporary improvement, but he suffered repeated relapses, with the involvement of new joints, and the treatment was finally discontinued.

The general impression derived from a study of the series of cases individually and as a whole is that inoculation was of material value in hastening recovery. The evidence is strongest in the more chronic ambulatory cases in which the benefit of rest in bed can be eliminated.

Certain other general therapeutic measures also contributed to the favorable results. The repeated withdrawal of joint fluid in large recurring effusions was no doubt of value. Only the smallest degree of fixation of joints was used. There were no cases in which ankylosis occurred. Prostatic massage has long been recognized as a valuable measure in gonococcus arthritis. It is highly probable on clinical grounds as well as from its effect in raising the opsonic index that prostatic massage results in an autoinoculation similar in its effect to the injection of killed gonococci. In several cases in which, for purposes of observation of the unaided effects of inoculation, systematic massage of the prostate was omitted, the condition of the prostate improved coincidentally with the improvement in the arthritis after inoculation.

SUMMARY.

Systemic infections by the gonococcus tend to spontaneous recovery. In many of the cases of gonococcus arthritis the clinical manifestations

are acute and the course extends over a few days or weeks. Certain cases, however, do not recover so quickly and the condition passes into a chronic stage which may last months or years. In the first class of cases immunity develops rapidly, and clinically little benefit appears to result from the injections of dead gonococci. In the chronic cases the mechanism of immunity fails to rid the body of the organisms, and they persist either in the original lesions or in one or more foci, such as the prostate, and give rise from time to time to new lesions at various points of localization. When dead gonococci are injected a reaction frequently follows, with an increase in constitutional and local symptoms. The reaction is later followed by a period of improvement. The fact that with a dose of constant size the reactions become less with each injection would seem to be a strong argument in favor of the value of the injections in hastening the development of immunity. Clinically in a number of cases the injections of dead gonococci have seemed to be of distinct value.

Many more series of cases will have to be studied before a definite opinion can be expressed, but the results obtained thus far seem to indicate that in certain cases of gonococcus arthritis recovery can be hastened by the injection of dead gonococci. No harm has been seen to follow the injections, and it is possible that the use of larger doses will be found desirable in some cases.

With further work the limitations as well as the advantages of the method will appear, and it should be recognized that while it is attractive theoretically as a specific therapeutic measure, too much must not be expected of it in the way of marvelous cures. It should be used rather in conjunction with other general measures, such as rest, aspiration of joints distended with fluid, massage of the prostate and other surgical and general hygienic treatment.

The reliability of the clinical gonococcus reaction as a diagnostic procedure will also be determined only after many tests. It has many points in common with the tuberculin reaction, and similarly, too, there may well be cases of gonococcus infection found which do not respond. It appears, however, to be well worth a trial. Should the reaction prove to be reliable, a valuable and much needed aid will be at hand for the diagnosis of obscure joint, synovial and periosteal diseases.

Until recently it has not been deemed expedient to carry on experiments on certain other phenomena of hypersusceptibility in gonococcus infections such as the ophthalmic reaction, though this work has now been begun. It has already been noted that the local reaction after

the injection of dead gonococci is greater in cases of gonococcus infection than in normal individuals.

I am greatly indebted to Dr. Hektoen of the Memorial Institute for advice and guidance during the preparation of this paper, and to the attending and house staffs of the Presbyterian and Cook County Hospitals for the opportunity afforded to observe clinical cases and for assistance in carrying out the treatment. I wish also to acknowledge the valuable assistance of Drs. A. H. Curtis and R. C. Menzies of the County Hospital, who devoted much time and care to the daily observation and recording of the clinical phenomena of the cases in their charge.

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